PATENT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

United States Patent and Trademark Office
(Box PCT)
Crystal Plaza 2 .
Washington, DC 20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 29 April 1999 (29.04.99)	
International application No. PCT/GB98/02383	**Applicant's or agent's file reference** SMW/LP5718911
International filing date (day/month/year) 07 August 1998 (07.08.98)	**Priority date (day/month/year)** 13 August 1997 (13.08.97)
Applicant HARBERD, Nicholas, Paul et al	

1. The designated Office is hereby notified of its election made:

    [X] in the demand filed with the International Preliminary Examining Authority on:

    08 March 1999 (08.03.99)

    [ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

    [ ] was not

    made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Lazar Joseph Panakal
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT 09/485529

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference SMW/LP5718911	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB98/02383	International filing date *(day/month/year)* 07/08/1998	Priority date *(day/month/year)* 13/08/1997

International Patent Classification (IPC) or national classification and IPC
C12N15/29

Applicant

PLANT BIOSCIENCE LIMITED et al.

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   I   ☒ Basis of the report
   II ☐ Priority
   III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   IV ☒ Lack of unity of invention
   V   ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   VI ☐ Certain documents cited
   VII ☐ Certain defects in the international application
   VIII ☒ Certain observations on the international application

Date of submission of the demand 08/03/1999	Date of completion of this report 15. 11. 99
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Meyer, W Telephone No. +49 89 2399 8157

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB98/02383

I. Basis of th report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-52   as originally filed

Claims, No.:

1-54   as originally filed

Drawings, sheets:

1/22-22/22   as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description,   pages:

☐ the claims,   Nos.:

☐ the drawings,   sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:

see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☒ all parts.

☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-5, 7, 10-13, 18-27, 32, 39-41, 44-50 and 52-54
	No:	Claims	6, 8,9, 14-17, 28-31, 33-38, 42, 43 and 51
Inventive step (IS)	Yes:	Claims	1-5, 7, 10-13 and 19-22
	No:	Claims	6, 8, 9, 14-18 and 23-54
Industrial applicability (IA)	Yes:	Claims	1-54
	No:	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

se separat sh et

Re Item IV

1.   The international search report has been drawn up in respect of the entire
      international application, but the IPEA finds that the application does not comply
      with the requirement of unity of invention (Article 34(3) and Rule 13 PCT).

2.   Reference is made to the following documents:

      D1:   Progress in Plant Breeding (ed. G.E. Russel), Pages 1-35, 1985
      D2:   Plant Cell, Vol. 5, March 1993, Pages 351-360
      D3:   FEBS Letters, Vol. 410, June 1997, Pages 213-218
      D4:   EMBL Accession No. D39460, 13 November 1994

      D1 was cited in the Application.

3.   An international application must relate to one invention only or to a group of
      inventions so linked as to form a single general inventive concept. Unity of
      invention is fulfilled only when there is a technical relationship between the
      inventions involving one or more of the same or corresponding special technical
      features, special technical features being such features, that define a contribution
      which each of the claimed inventions, considered **as a whole**, makes over the
      prior art.

4.   It might be argued, that the single inventive concept linking the claims together is
      that they all refer to a wheat- and maize-gibberellin responsive element. However,
      these complex set of claims are not at all limited only to the provision of either a
      wheat- or a maize-gibberellin responsive element (see e.g. claim 14 or claim 16).
      The only technical relationship between the independent claims is that these
      claims refer to genes responsible for dwarfism. This relation, however, cannot be
      accepted to consist of special technical feature as defined above since it does not
      define a contribution which each of the different claimed inventions, considered as
      a whole makes over the prior art.
      In fact, genes related to dwarfism have already been disclosed in the prior art: D1
      discloses dwarfing genes and their chromosomal localisation in wheat, which are
      gibberellin sensitive or insensitive. D1 does not disclose the molecular basis of

those genes. D2 discloses genes which are related to dwarfism in wheat). Thus, this technical feature cannot be accepted as a <u>special</u> technical feature as required by Rule 13 PCT.

5. Consequently, the technical relationship of the independent claims does not embrace a special technical feature, within the meaning of Rule 13.2 PCT. The following "potential inventions" have to be considered separately:

   1. The subject-matter of **claims 1-9 and partially 10, 12, 14, 15, 17, 18, 21-23, 28-54**, refer to a gene related to dwarfism in wheat, which encodes a polypeptide comprising the amino acids sequence DELLAALGYKVRASDMA.

   2. The subject-matter of **claims 16, 26, 27 and partially 28-54** refer to a gene related to dwarfism in wheat, which encodes a polypeptide in which the amino acids sequence DELLAALGYKVRASDMA is deleted.

   3. The subject-matter of **claims 11, 13 and partially 10, 12, 14, 15, 17, 18, 21-23 and 28-54,** refer to a gene related to dwarfism in wheat, which encodes a polypeptide comprising the amino acids sequence DELLAALGYKVR<u>SS</u>DMA

   4. The subject-matter of **claims 19, 20, 24, 25 and partially 28-54** refer to a gene related to dwarfism in wheat, which encodes a polypeptide in which the amino acids sequence DELLAALGYKVR<u>SS</u>DMA is deleted.

6. The technical contributions of these 4 groups of claims are different and are not directly linked or corresponding so that they could be considered to contribute a special technical feature as defined in Rule 13 PCT. Hence, the application lacks unity under Article 34(3) PCT in the light of Rule 13.2 PCT.

Re Item V

7. As the priority document for the application was not available at the time of drafting of the present written opinion, it is established under the assumption that the entire subject-matter is entitled to the claimed priority. If this is not the case, the 'P' document cited in the search report may have to be considered for novelty and/or inventive step. These documents might be detrimental to most, if not all, of the claimed subject-matter (Article 33(2), (3) PCT).

8. The subject-matter of **claims 6, 8, 9, 14-17, 28-31 and 51** is not new in the sense of Article 33(2) PCT. The subject-matter is anticipated by D3. This document discloses a cDNA cloned into an inducible vector and its corresponding amino acid sequence. The nucleotide sequence has at least 80% identity with nucleotide sequence disclosed in present claim 6. The amino acids sequence of D3 has at least 16 amino acid residues, which are similar to the sequence shown in present claim 8. D3 further discloses a polynucleotide encoding a polypeptide which discloses at least 80% similarity to the amino acid sequence of the Rht polypeptide of Triticum Aestivum in which also the amino acid sequence disclosed in **claims 16 and 17** is not present.

The argument that a functional feature of a claimed product limits this product in such a way as to distinguish it clearly form a product disclosed in the prior art, in order to established novelty cannot be followed. A known product (here a polypeptide) which prima facie is the same as the substance defined in the claim and which is in a form which is in fact suitable for the stated use, though it has never been described for that use, deprives the claim of novelty (see PCT, Guidelines 4.8). Thus, the additional functional characterising features like e.g. "inhibition of growth", "gibberellin-unresponsive dwarfism" or "suitable for use in antisense or sense regulation" do not bestow these claims with novelty (see PCT Guidelines 4.8). In consequence, D3 takes away the novelty of present **claims 6, 8, 9, 14-17, 28-31 and 51**.

9. The subject-matter of **claims 18-23, 32-49 and 52-54** are concerned with matter which can be obtained in an obvious manner from the material (polynucleotide sequence) disclosed in D3 (see also item 8., above). Once new plant cDNAs are available, the subcloning of these cDNAs into a suitable transformation vector and to transform these vectors into plants would be a matter of mere routine for the skilled person in the specific field. Furthermore, the creation of antibodies, is also mere routine performance for a person skilled in the art. Hence, no inventive step is present in the subject-matter of **claims 32-49 and 52-54** (Article 33(3) PCT).

10. The subject-matter of **claims 33-38, 42 and 43** is not new in the sense of Article 33(2) PCT. D1 discloses the introduction of semi-dwarfing Rht homeoalleles into elite bread-wheat breeding lines. In other words D1 discloses the introduction of a "heterologous Rht" gene into wheat. It is herby noted that the term "heterologous"

as used in **claim 33** indicates that the gene/sequence of nucleotides in question have been introduced into said cell of the plant, or an ancestor thereof, using genetic engineering, which includes also crossings. It might be argued, that the EPO makes a distinction between selective breeding and genetic engineering, in that the former is explicitly considered to be essentially biological and therefore excluded from patentability. However, this argumentation is not relevant for the assessment of novelty or inventive activity. Even a subject-matter, which is excluded from patentability is considered to be state of the art. Consequently, the subject- matter of **claims 33-38, 42 and 43** does not fulfil the requirements of Article 33(2) PCT.

11. The subject-matter of **claim 50** is dependent on the **claim 49**. The subject-matter of this claim does not appear to include any additional matter which would render it inventive as such. It appears to be concerned with mere technical variations of the known subject-matter of **claim 50**. It appears that they would be allowable only in combination with a novel and inventive main claim.

12. With respect to the documents cited in the International Search Report, the claimed subject-matter of **claims 1-5, 7, 19-22, 26 and 27** fulfill the requirement of Article 33(2) PCT.
The subject-matter of **claims 1-5, 7, 19-22, 26 and 27** also fulfill the requirement of Article 33(3) PCT.
D1 is considered to represent the closest prior art document, which discloses the D1 discloses the introduction of semi-dwarfing Rht homeoalleles into elite bread-wheat breeding lines.
Claims 1-5, 7, 19-22, 26 and 27 are distinguished therefrom by disclosing a gibberellin responsive element, responsible for inhibition of growth in a plant.
The technical problem to be solved by the present application was therefore to provide the gibberellin responsive element.
The solution to this problem proposed in **claims 1-5, 7, 19-22, 26 and 27** of the present application is considered as involving an inventive step (Article 33(3) PCT) as a person skilled in the specific field could not directly deduce in an obvious manner from the available prior art the element in wheat which is responsible for inhibition the growth and which is in addition gibberellin inducible. Consequently, **claims 1-5, 7, 19-22, 26 and 27** appear to meet the requirements

of Article 33(3) PCT.

13. With respect to the documents cited in the International Search Report, the claimed subject-matter of **claims 10-13, 24 and 25** fulfill the requirement of Article 33(2) PCT.

The subject-matter of **claims 10-13, 24 and 25** also fulfill the requirement of Article 33(3) PCT.

D2 is considered to represent the closest prior art document, which discloses the genetic analysis of gibberellin insensitive Arabidopsis mutations.

Claims 10-13, 24 and 25 are distinguished therefrom by disclosing the Arabidopsis gibberellin responsive element, responsible for inhibition of growth in Arabidopsis.

The technical problem to be solved by the present application was therefore to provide the gibberellin responsive element of Arabidopsis.

The solution to this problem proposed in **claims 10-13, 24 and 25** of the present application is considered as involving an inventive step (Article 33(3) PCT) as a person skilled in the specific field could not directly deduce in an obvious manner from the available prior art the element in Arabidopsis which is responsible for inhibition the growth and which is in addition gibberellin inducible.

Consequently, the subject-matter of **claims 10-13, 24 and 25** appears to meet the requirements of Article 33(3) PCT.

Re Item VIII

14. The expression "specifically hybridise" are vague and not suitable to clearly define the scope of e.g. **claims 48 or 52**. Without any indication of the reaction condition to be used for hybridisation this is an essentially undefined term which is open to individual interpretation (Article 6 PCT). It might be argued that the description on page 21, line 23 to page 25, line 14 includes conditions that might be use for hybridisation, however there this does not clearly define the wording "specifically".

15. DNA sequence encoding a protein (see e.g. **claim 14**) - being a chemical product - has to be characterised e.g. by its sequence or as a product by process, but not merely by its function. According to the PCT-Guidelines C III 4.7. and 4.7.a, the area defined by the claims must be as precise as the invention allows. That

means that claims which attempt to define the invention, or a feature thereof, by a result to be achieved, should not be allowed.

Similar a protein being a chemical product should be characterised by technical features and not by simply naming. Thus the term "Rht-polypeptide" is not a recognized suitable to clarify the scope of the claims. A corresponding SEQ ID NO. should be added.

16. The subject-matter of **claims 19-20 and 24-27** is not supported by the description as required by Article 6 PCT, as their scope is broader than justified by the description. The reasons are the following: The essential feature for this application is that the expression of nucleotide sequence encoding the amino acids sequence DELLAALGYKVRASDMA (or DELLAALGYKVRASSMA) in a plant provides inhibition of growth, which inhibition is antagonised by gibberellin. However, the subject-matter of **claims 19-20 and 24-27** excludes the above mentioned essential feature. Furthermore, no experimental support can be found in the description that on expression of nucleotide sequence encoding the amino acids sequence DELLAALGYKVRASDMA (or DELLAALGYKVRASSMA), in which this sequence is deleted provides inhibition of growth, which inhibition is antagonised by gibberellin. Your attention is drawn to the following, if in a later phase the Applicant could demonstrate that the expression of a nucleotide in which the amino acid above mentioned sequence is deleted would indeed provide inhibition of growth which inhibition is antagonised by gibberellin, than it appears that the subject-matter of the remaining claims would not fulfil the requirements of Article 6 PCT.

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(54) Title: GENETIC CONTROL OF PLANT GROWTH AND DEVELOPMENT

(57) Abstract

The wheat *Rht* gene and homologues from other species including rice and maize (the *D8* gene), useful for modification of growth and/or development characteristics of plants. Transgenic plants and methods and means for their production.

GENETIC CONTROL OF PLANT GROWTH AND DEVELOPMENT

This invention relates to the genetic control of growth
and/or development of plants and the cloning and expression

5 of genes involved therein. More particularly, the invention
relates to the cloning and expression of the *Rht* gene of
Triticum Aestivum, and homologues from other species, and use
of the genes in plants.

10 An understanding of the genetic mechanisms which influence
growth and development of plants, including flowering,
provides a means for altering the characteristics of a target
plant. Species for which manipulation of growth and/or
development characteristics may be advantageous includes all

15 crops, with important examples being the cereals, rice and
maize, probably the most agronomically important in warmer
climatic zones, and wheat, barley, oats and rye in more
temperate climates. Important crops for seed products are
oil seed rape and canola, maize, sunflower, soyabean and

20 sorghum. Many crops which are harvested for their roots are,
of course, grown annually from seed and the production of
seed of any kind is very dependent upon the ability of the
plant to flower, to be pollinated and to set seed. In
horticulture, control of the timing of growth and

25 development, including flowering, is important.
Horticultural plants whose flowering may be controlled
include lettuce, endive and vegetable brassicas including
cabbage, broccoli and cauliflower, and carnations and

geraniums. Dwarf plants on the one hand and over-size,
taller plants on the other may be advantageous and/or
desirable in various horticultural and agricultural contexts,
further including trees, plantation crops and grasses.

5

Recent decades have seen huge increases in wheat grain yields
due to the incorporation of semi-dwarfing *Rht* homeoalleles
into breeding programmes. These increases have enabled wheat
productivity to keep pace with the demands of the rising

10 world population. Previously, we described the cloning of
the *Arabidopsis gai* alleles (International patent application
PCT/GB97/00390 filed 12 February 1997 and published as
WO97/29123 on 14 August 1998, John Innes Centre Innovations
Limited, the full contents of which are incorporated herein

15 by reference) which, like *Rht* mutant alleles in wheat (a
monocot), confers a semi-dominant dwarf phenotype in
Arabidopsis (a dicot) and a reduction in responsiveness to
the plant growth hormone gibberellin (GA). *gai* encodes a
mutant protein (gai) which lacks a 17 amino acid residue

20 segment found near the N-terminus of the wild-type (GAI)
protein. The sequence of this segment is highly conserved in
a rice cDNA sequence (EST). Here we show that this cDNA maps
to a short section of the overlapping cereal genome maps
known to contain the *Rht* loci, and that we have used the cDNA

25 to isolate the *Rht* genes of wheat. That genomes as widely
diverged as those of *Arabidopsis* and *Triticum* should carry a
conserved sequence which, when mutated, affects GA
responsiveness, indicates a role for that sequence in GA

signalling that is conserved throughout the plant kingdom.
Furthermore, cloning of *Rht* permits its transfer to many
different crop species, with the aim of yield enhancement as
great as that obtained previously with wheat.

5

The introduction of semi-dwarfing *Rht* homeoalleles
(originally known as Norin 10 genes, derived from a Japanese
variety, Norin 10) into elite bread-wheat breeding lines was
one of the most significant contributors to the so-called

10 "green revolution" (Gale *et al*, 1985. Dwarfing genes in
wheat. In: Progress in Plant Breeding, G.E. Russell (ed)
Butterworths, London pp 1-35). Wheat containing these
homeoalleles consistently out-yield wheats lacking them, and
now comprise around 80% of the world's wheat crop. The

15 biological basis of this yield-enhancement appears to be two-
fold. Firstly, the semi-dwarf phenotype conferred by the *Rht*
alleles causes an increased resistance to lodging (flattening
of plants by wind/rain with consequent loss of yield).
Secondly, these alleles cause a reallocation of

20 photoassimilate, with more being directed towards the grain,
and less towards the stem (Gale *et al*, 1985). These
properties have major effects on wheat yields, as
demonstrated by the fact that UK wheat yields increased by
over 20% during the years that *Rht*-containing lines were

25 taken up by farmers.

The *rht* mutants are dwarfed because they contain a
genetically dominant, mutant *rht* allele which compromises

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their responses to gibberellin (GA, an endogenous plant
growth regulator) (Gale *et al*, 1976. Heredity 37; 283-289).
Thus the coleoptiles of *rht* mutants, unlike those of wild-
type wheat plants, do not respond to GA applications. In
5 addition, *rht* mutants accumulate biologically active GAs to
higher levels than found in wild-type controls (Lenton *et al*,
1987. Gibberellin insensitivity and depletion in wheat -
consequences for development. In: Hormone action in Plant
Development - a critical appraisal. GV Haod, JR Lenton, MB
10 Jackson and RK Atkin (eds) Butterworths, London pp 145-160).
These properties (genetic dominance, reduced GA-responses,
and high endogenous GA levels) are common to the phenotypes
conferred by mutations in other species (*D8/D9* in maize; *gai*
in *Arabidopsis*), indicating that these mutant alleles define
15 orthologous genes in these different species, supported
further by the observation that *D8/D9* and *Rht* are syntenous
loci in the genomes of maize and wheat.

According to a first aspect of the present invention there is
20 provided a nucleic acid molecule comprising a nucleotide
sequence encoding a polypeptide with Rht function. The term
"Rht function" indicates ability to influence the phenotype
of a plant like the *Rht* gene of *Triticum*. "*Rht* function" may
be observed phenotypically in a plant as inhibition,
25 suppression, repression or reduction of plant growth which
inhibition, suppression, repression or reduction is
antagonised by GA. *Rht* expression tends to confer a dwarf
phenotype on a plant which is antagonised by GA.

Overexpression in a plant from a nucleotide sequence encoding
a polypeptide with *Rht* function may be used to confer a dwarf
phenotype on a plant which is correctable by treatment with
GA.

5

Also according to an aspect of the present invention there is
provided a nucleic acid molecule comprising a nucleotide
sequence encoding a polypeptide with ability to confer a *rht*
mutant phenotype upon expression. *rht* mutant plants are
10 dwarfed compared with wild-type, the dwarfing being GA-
insensitive.

Herein, "Rht" (capitalised) is used to refer to the wild-type
function, while "rht" (uncapitalised) is used to refer to
15 mutant function.

Many plant growth and developmental processes are regulated
by specific members of a family of tetracyclic diterpenoid
growth factors known as gibberellins (GA) (Hooley, *Plant Mol.*
20 *Biol.* 26, 1529-1555 (1994)). By gibberellin or GA is meant a
diterpenoid molecule with the basic carbon-ring structure
shown in Figure 5 and possessing biological activity, i.e. we
refer to biologically active gibberellins.

25 Biological activity may be defined by one or more of
stimulation of cell elongation, leaf senescence or
elicitation of the cereal aleurone α-amylase response. There
are many standard assays available in the art, a positive

result in any one or more of which signals a test gibberellin
as biologically active (Hoad et al., *Phytochemistry* 20, 703-
713 (1981); Serebryakov et al., *Phytochemistry* 23, 1847-1854
(1984); Smith et al., *Phytochemistry* 33, 17-20 (1993)).

5

Assays available in the art include the lettuce hypocotyl
assay, cucumber hypocotyl assay, and oat first leaf assay,
all of which determine biological activity on the basis of
ability of an applied gibberellin to cause elongation of the
10 respective tissue. Preferred assays are those in which the
test composition is applied to a gibberellin-deficient plant.
Such preferred assays include treatment of dwarf GA-deficient
Arabidopsis to determine growth, the dwarf pea assay, in
which internode elongation is determined, the Tan-ginbozu
15 dwarf rice assay, in which elongation of leaf sheath is
determined, and the *d5*-maize assay, also in which elongation
of leaf sheath is determined. The elongation bioassays
measure the effects of general cell elongation in the
respective organs and are not restricted to particular cell
20 types.

Further available assays include the dock (*Rumex*) leaf
senescence assay and the cereal aleurone α-amylase assay.
Aleurone cells which surround the endosperm in grain secrete
25 α-amylase on germination, which digests starch to produce
sugars then used by the growing plant. The enzyme production
is controlled by GA. Isolated aleurone cells given
biologically active GA secrete α-amylase whose activity can

then be assayed, for example by measurement of degradation of
starch.

Structural features important for high biological activity
5 (exhibited by GA_1, GA_3, GA_4 and GA_7) are a carboxyl group on
C-6 of B-ring; C-19, C-10 lactone; and β-hydroxylation at C-
3. β-hydroxylation at C-2 causes inactivity (exhibited by
GA_8, GA_{29}, GA_{34} and GA_{51}).   *rht* mutants do not respond to GA
treatment, e.g. treatment with GA_1, GA_3 or GA_4.
10

Treatment with GA is preferably by spraying with aqueous
solution, for example spraying with 10^{-4}M GA_3 or GA_4 in
aqueous solution, perhaps weekly or more frequently, and may
be by placing droplets on plants rather than spraying.   GA
15 may be applied dissolved in an organic solvent such as
ethanol or acetone, because it is more soluble in these than
in water, but this is not preferred because these solvents
have a tendency to damage plants.   If an organic solvent is
to be used, suitable formulations include 24ηl of 0.6, 4.0 or
20 300mM GA_3 or GA_4 dissolved in 80% ethanol.   Plants, e.g.
Arabidopsis, may be grown on a medium containing GA, such as
tissue culture medium (GM) solidified with agar and
containing supplementary GA.

25 Nucleic acid according to the present invention may have the
sequence of a wild-type *Rht* gene of *Triticum* or be a mutant,
derivative, variant or allele of the sequence provided.
Preferred mutants, derivatives, variants and alleles are

those which encode a protein which retains a functional
characteristic of the protein encoded by the wild-type gene,
especially the ability for plant growth inhibition, which
inhibition is antagonised by GA, or ability to confer on a

5 plant one or more other characteristics responsive to GA
treatment of the plant. Other preferred mutants,
derivatives, variants and alleles encode a protein which
confers a *rht* mutant phenotype, that is to say reduced plant
growth which reduction is insensitive to GA, i.e. not

10 overcome by GA treatment. Changes to a sequence, to produce
a mutant, variant or derivative, may be by one or more of
addition, insertion, deletion or substitution of one or more
nucleotides in the nucleic acid, leading to the addition,
insertion, deletion or substitution of one or more amino

15 acids in the encoded polypeptide. Of course, changes to the
nucleic acid which make no difference to the encoded amino
acid sequence are included.

A preferred nucleotide sequence for a *Rht* gene is one which

20 encodes the RHT amino acid sequence shown in Figure 3b,
especially a *Rht* coding sequence shown in Figure 3a. A
preferred *rht* mutant lacks part or all of the 17 amino acid
sequence underlined in Figure 3b, and/or part or the sequence
DVAQKLEQLE, which immediately follows the 17 amino acid

25 sequence underlined in Figure 3b.

Further preferred nucleotide sequences encode the amino acid
sequence shown in any other figure herein, especially a

coding sequence shown in a Figure. Further embodiments of
the present invention, in all aspects, employ a nucleotide
sequence encoding the amino acid sequence shown in Figure 6b,
7b, 8b, 9b, 11b, 11d or 12b. Such a coding sequence may be
5 as shown in Figure 6a, 7a, 8a, 9a, 11a, 11c or 12a.

The present invention also provides a nucleic acid construct
or vector which comprises nucleic acid with any one of the
provided sequences, preferably a construct or vector from
10 which polypeptide encoded by the nucleic acid sequence can be
expressed. The construct or vector is preferably suitable
for transformation into a plant cell. The invention further
encompasses a host cell transformed with such a construct or
vector, especially a plant cell. Thus, a host cell, such as
15 a plant cell, comprising nucleic acid according to the
present invention is provided. Within the cell, the nucleic
acid may be incorporated within the chromosome. There may be
more than one heterologous nucleotide sequence per haploid
genome. This, for example, enables increased expression of
20 the gene product compared with endogenous levels, as
discussed below.

A construct or vector comprising nucleic acid according to
the present invention need not include a promoter or other
25 regulatory sequence, particularly if the vector is to be used
to introduce the nucleic acid into cells for recombination
into the genome. However, in one aspect the present
invention provides a nucleic acid construct comprising a *Rht*

or *rht* coding sequence (which includes homologues from other
than *Triticum*) joined to a regulatory sequence for control of
expression, the regulatory sequence being other than that
naturally fused to the coding sequence and preferably of or
5 derived from another gene.

Nucleic acid molecules and vectors according to the present
invention may be as an isolate, provided isolated from their
natural environment, in substantially pure or homogeneous
10 form, or free or substantially free of nucleic acid or genes
of the species of interest or origin other than the sequence
encoding a polypeptide able to influence growth and/or
development, which may include flowering, eg in *Triticum
Aestivum* nucleic acid other than the *Rht* coding sequence.
15 The term "nucleic acid isolate" encompasses wholly or
partially synthetic nucleic acid.

Nucleic acid may of course be double- or single-stranded,
cDNA or genomic DNA, RNA, wholly or partially synthetic, as
20 appropriate. Of course, where nucleic acid according to the
invention includes RNA, reference to the sequence shown
should be construed as encompassing the RNA equivalent, with
U substituted for T.

25 The present invention also encompasses the expression product
of any of the nucleic acid sequences disclosed and methods of
making the expression product by expression from encoding
nucleic acid therefor under suitable conditions in suitable

11

host cells. Those skilled in the art are well able to

construct vectors and design protocols for expression and

recovery of products of recombinant gene expression.

Suitable vectors can be chosen or constructed, containing

5 appropriate regulatory sequences, including promoter

sequences, terminator fragments, polyadenylation sequences,

enhancer sequences, marker genes and other sequences as

appropriate. For further details see, for example, *Molecular*

Cloning: a Laboratory Manual: 2nd edition, Sambrook et al,

10 1989, Cold Spring Harbor Laboratory Press. Transformation

procedures depend on the host used, but are well known. Many

known techniques and protocols for manipulation of nucleic

acid, for example in preparation of nucleic acid constructs,

mutagenesis, sequencing, introduction of DNA into cells and

15 gene expression, and analysis of proteins, are described in

detail in *Protocols in Molecular Biology*, Second Edition,

Ausubel et al. eds., John Wiley & Sons, 1992. Specific

procedures and vectors previously used with wide success upon

plants are described by Bevan, Nucl. Acids Res. (1984) 12,

20 8711-8721), and Guerineau and Mullineaux, (1993) Plant

transformation and expression vectors. In: Plant Molecular

Biology Labfax (Croy RRD ed) Oxford, BIOS Scientific

Publishers, pp 121-148. The disclosures of Sambrook et al.

and Ausubel et al. and all other documents mentioned herein

25 are incorporated herein by reference.

Expression as a fusion with a polyhistidine tag allows

purification of Rht or rht to be achieved using Ni-NTA resin

available from QIAGEN Inc. (USA) and DIAGEN GmbH (Germany).

See Janknecht et al., *Proc. Natl. Acad. Sci. USA* 88, 8972-

8976 (1991) and EP-A-0253303 and EP-A-0282042. Ni-NTA resin

has high affinity for proteins with consecutive histidines

5 close to the N- or C- terminus of the protein and so may be

used to purifiy histidine-tagged Rht or rht proteins from

plants, plant parts or extracts or from recombinant organisms

such as yeast or bacteria, e.g. *E. coli*, expressing the

protein.

10

Purified Rht protein, e.g. produced recombinantly by

expression from encoding nucleic acid therefor, may be used

to raise antibodies employing techniques which are standard

in the art. Antibodies and polypeptides comprising antigen-

15 binding fragments of antibodies may be used in identifying

homologues from other species as discussed further below.

Methods of producing antibodies include immunising a mammal

(eg human, mouse, rat, rabbit, horse, goat, sheep or monkey)

20 with the protein or a fragment thereof. Antibodies may be

obtained from immunised animals using any of a variety of

techniques known in the art, and might be screened,

preferably using binding of antibody to antigen of interest.

For instance, Western blotting techniques or

25 immunoprecipitation may be used (Armitage et al, 1992, Nature

357: 80-82). Antibodies may be polyclonal or monoclonal.

As an alternative or supplement to immunising a mammal,

13

antibodies with appropriate binding specificty may be

obtained from a recombinantly produced library of expressed

immunoglobulin variable domains, eg using lambda

bacteriophage or filamentous bacteriophage which display

5 functional immunoglobulin binding domains on their surfaces;

for instance see WO92/01047.

Antibodies raised to a Rht, or rht, polypeptide can be used

in the identification and/or isolation of homologous

10 polypeptides, and then the encoding genes. Thus, the present

invention provides a method of identifying or isolating a

polypeptide with Rht function or ability to confer a *rht*

mutant phenotype, comprising screening candidate polypeptides

with a polypeptide comprising the antigen-binding domain of

15 an antibody (for example whole antibody or a fragment

thereof) which is able to bind an *Triticum Aestivum* Rht or

rht polypeptide, or preferably has binding specificity for

such a polypeptide, such as having the amino acid sequence

shown in Figure 3b.

20

Candidate polypeptides for screening may for instance be the

products of an expression library created using nucleic acid

derived from an plant of interest, or may be the product of a

purification process from a natural source.

25

A polypeptide found to bind the antibody may be isolated and

then may be subject to amino acid sequencing. Any suitable

technique may be used to sequence the polypeptide either

wholly or partially (for instance a fragment of the
polypeptide may be sequenced). Amino acid sequence
information may be used in obtaining nucleic acid encoding
the polypeptide, for instance by designing one or more
5 oligonucleotides (e.g. a degenerate pool of oligonucleotides)
for use as probes or primers in hybridisation to candidate
nucleic acid, as discussed further below.

A further aspect of the present invention provides a method
10 of identifying and cloning *Rht* homologues from plant species
other than *Triticum* which method employs a nucleotide
sequence derived from any shown in Figure 2 or Figure 3a, or
other figure herein. Sequences derived from these may
themselves be used in identifying and in cloning other
15 sequences. The nucleotide sequence information provided
herein, or any part thereof, may be used in a data-base
search to find homologous sequences, expression products of
which can be tested for *Rht* function. Alternatively, nucleic
acid libraries may be screened using techniques well known to
20 those skilled in the art and homologous sequences thereby
identified then tested.

For instance, the present invention also provides a method of
identifying and/or isolating a *Rht* or *rht* homologue gene,
25 comprising probing candidate (or "target") nucleic acid with
nucleic acid which encodes a polypeptide with *Rht* function or
a fragment or mutant, derivative or allele thereof. The
candidate nucleic acid (which may be, for instance, cDNA or

genomic DNA) may be derived from any cell or organism which
may contain or is suspected of containing nucleic acid
encoding such a homologue.

5 In a preferred embodiment of this aspect of the present
invention, the nucleic acid used for probing of candidate
nucleic acid encodes an amino acid sequence shown in Figure
3b, a sequence complementary to a coding sequence, or a
fragment of any of these, most preferably comprising a
10 nucleotide sequence shown in Figure 3a.

Alternatively, as discussed, a probe may be designed using
amino acid sequence information obtained by sequencing a
polypeptide identified as being able to be bound by an
15 antigen-binding domain of an antibody which is able to bind a
Rht or rht polypeptide such as one with the Rht amino acid
sequence shown in Figure 3b.

Preferred conditions for probing are those which are
20 stringent enough for there to be a simple pattern with a
small number of hybridizations identified as positive which
can be investigated further. It is well known in the art to
increase stringency of hybridisation gradually until only a
few positive clones remain.

25

As an alternative to probing, though still employing nucleic
acid hybridisation, oligonucleotides designed to amplify DNA
sequences from Rht genes may be used in PCR or other methods

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involving amplification of nucleic acid, using routine

procedures. See for instance "PCR protocols; A Guide to

Methods and Applications", Eds. Innis et al, 1990, Academic

Press, New York.

5

Preferred amino acid sequences suitable for use in the design

of probes or PCR primers are sequences conserved (completely,

substantially or partly) between *Rht* genes.

10 On the basis of amino acid sequence information,

oligonucleotide probes or primers may be designed, taking

into account the degeneracy of the genetic code, and, where

appropriate, codon usage of the organism from which the

candidate nucleic acid is derived. In particular, primers

15 and probes may be designed using information on conserved

sequences apparent from, for example, Figure 3 and/or Figure

4, also Figure 10.

Where a full-length encoding nucleic acid molecule has not

20 been obtained, a smaller molecule representing part of the

full molecule, may be used to obtain full-length clones.

Inserts may be prepared for example from partial cDNA clones

and used to screen cDNA libraries. The full-length clones

isolated may be subcloned into vectors such as expression

25 vectors or vectors suitable for transformation into plants.

Overlapping clones may be used to provide a full-length

sequence.

The present invention also extends to nucleic acid encoding

Rht or a homologue obtainable using a nucleotide sequence

derived from Figure 2 or Figure 3a, and such nucleic acid

obtainable using one or more, e.g. a pair, of primers

5 including a sequence shown in Table 1.

Also included within the scope of the present invention are

nucleic acid molecules which encode amino acid sequences

which are homologues of the polypeptide encoded by *Rht* of

10 *Triticum.* A homologue may be from a species other than

Triticum.

Homology may be at the nucleotide sequence and/or amino acid

sequence level. Preferably, the nucleic acid and/or amino

15 acid sequence shares homology with the sequence encoded by

the nucleotide sequence of Figure 3a, preferably at least

about 50%, or 60%, or 70%, or 80% or 85% homology, most

preferably at least 90%, 92%, 95% or 97% homology. Nucleic

acid encoding such a polypeptide may preferably share with

20 the *Triticum Rht* gene the ability to confer a particular

phenotype on expression in a plant, preferably a phenotype

which is GA responsive (i.e. there is a change in a

characteristic of the plant on treatment with GA), such as

the ability to inhibit plant growth where the inhibition is

25 antagonised by GA. As noted, *Rht* expression in a plant may

affect one or more other characteristics of the plant. A

preferred characteristic that may be shared with the *Triticum*

Rht gene is the ability to complement a *Rht* null mutant

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phenotype in a plant such as *Triticum*, such phenotype being

resistance to the dwarfing effect of paclobutrazol. The

slender mutant of barley maps to a location in the barley

genome equivalent to that of *Rht* in the wheat genome. Such

5 mutant plants are strongly paclobutrazol resistant. The

present inventors believe that the *slender* barley mutant is a

null mutant allele of the orthologous gene to wheat *Rht*,

allowing for complementation of the barley mutant with the

wheat gene. Ability to complement a *slender* mutant in barley

10 may be a characteristic of embodiments of the present

invention.

Some preferred embodiments of polypeptides according to the

present invention (encoded by nucleic acid embodiments

15 according to the present invention) include the 17 amino acid

sequence which is underlined in Figure 3b, or a contiguous

sequence of amino acids residues with at least about 10

residues with similarity or identity with the respective

corresponding residue (in terms of position) in 17 amino

20 acids which are underlined in Figure 3b, more preferably 11,

12, 13, 14, 15, 16 or 17 such residues, and/or the sequence

DVAQKLEQLE, or a contiguous sequence of amino acids with at

least about 5 residues with similarity or identity with the

respective corresponding residue (in terms of position)

25 within DVAQKLEQLE, more preferably 6, 7, 8 or 9 such

residues. Further embodiments include the 27 amino acid

sequence DELLAALGYKVRASDMADVAQKLEQLE, or a contiguous

sequence of amino acids residues with at least about 15

19

residues with similarity or identity with the respective

corresponding residue (in terms of position) within this

sequence, more preferably 16, 17, 18, 19, 20, 21, 22, 23, 24,

25 or 26 such residues.

5

As is well-understood, homology at the amino acid level is

generally in terms of amino acid similarity or identity.

Similarity allows for "conservative variation", i.e.

substitution of one hydrophobic residue such as isoleucine,

10 valine, leucine or methionine for another, or the

substitution of one polar residue for another, such as

arginine for lysine, glutamic for aspartic acid, or glutamine

for asparagine. Similarity may be as defined and determined

by the TBLASTN program, of Altschul *et al*. (1990) *J. Mol.*

15 *Biol*. 215: 403-10, which is in standard use in the art, or

more preferably GAP (Program Manual for the Wisconsin

Package, Version 8, September 1994, Genetics Computer Group,

575 Science Drive, Madison, USA), which uses the algorithm of

Needleman and Wunsch to align sequences. Suitable parameters

20 for GAP include the default parameters, a gap creation

penalty = 12 and gap extension penalty = 4, or gap creation

penalty 3.00 and gap extension penalty 0.1. Homology may be

over the full-length of the *Rht* sequence of Figure 3b, or may

more preferably be over a contiguous sequence of 10 amino

25 acids compared with DVAQKLEQLE, and/or a contiguous sequence

of 17 amino acids, compared with the 17 amino acids

underlined in Figure 3b, and/or a contiguous sequence of 27

amino acids compared with DELLAALGYKVRASDMADVAQKLEQLE, or a

longer sequence, e.g. about 30, 40, 50 or more amino acids,

compared with the amino acid sequence of Figure 3b and

preferably including the underlined 17 amino acids and/or

DVAQKLEQLE.

5

At the nucleic acid level, homology may be over the full-

length or more preferably by comparison with the 30

nucleotide coding sequence within the sequence of Figure 3a

and encoding the sequence DVAQKLEQLE and/or the 51 nucleotide

10   coding sequence within the sequence of Figure 3a and encoding

the 17 amino acid sequence underlined in Figure 3b, or a

longer sequence, e.g. about, 60, 70, 80, 90, 100, 120, 150 or

more nucleotides and preferably including the 51 nucleotide

of Figure 3 which encodes the underlined 17 amino acid

15   sequence of Figure 3b.

As noted, similarity may be as defined and determined by the

TBLASTN program, of Altschul *et al.* (1990) *J. Mol. Biol.* 215:

403-10, which is in standard use in the art, or the standard

20   program BestFit, which is part of the Wisconsin Package,

Version 8, September 1994, (Genetics Computer Group, 575

Science Drive, Madison, Wisconsin, USA, Wisconsin 53711).

BestFit makes an optimal alignment of the best segment of

similarity between two sequences. Optimal alignments are

25   found by inserting gaps to maximize the number of matches

using the local homology algorithm of Smith and Waterman

(*Adv. Appl. Math.* (1981) 2: 482-489). Other algorithms

include GAP, which uses the Needleman and Wunsch algorithm to

21

align two complete sequences that maximizes the number of
matches and minimizes the number of gaps. As with any
algorithm, generally the default parameters are used, which
for GAP are a gap creation penalty = 12 and gap extension
5 penalty = 4. The algorithm FASTA (which uses the method of
Pearson and Lipman (1988) *PNAS USA* 85: 2444-2448) is a
further alternative.

Use of either of the terms "homology" and "homologous" herein
10 does not imply any necessary evolutionary relationship
between compared sequences, in keeping for example with
standard use of terms such as "homologous recombination"
which merely requires that two nucleotide sequences are
sufficiently similar to recombine under the appropriate
15 conditions. Further discussion of polypeptides according to
the present invention, which may be encoded by nucleic acid
according to the present invention, is found below.

The present invention extends to nucleic acid that hybridizes
20 with any one or more of the specific sequences disclosed
herein under stringent conditions.

Hybridisation may be be determined by probing with nucleic
acid and identifying positive hybridisation under suitably
25 stringent conditions (in accordance with known techniques).
For probing, preferred conditions are those which are
stringent enough for there to be a simple pattern with a
small number of hybridisations identified as positive which

22

can be investigated further.   It is well known in the art to

increase stringency of hybridisation gradually until only a

few positive clones remain.

5   Binding of a probe to target nucleic acid (e.g. DNA) may be

measured using any of a variety of techniques at the disposal

of those skilled in the art.   For instance, probes may be

radioactively, fluorescently or enzymatically labelled.

Other methods not employing labelling of probe include

10 examination of restriction fragment length polymorphisms,

amplification using PCR, RNAase cleavage and allele specific

oligonucleotide probing.

Probing may employ the standard Southern blotting technique.

15 For instance DNA may be extracted from cells and digested

with different restriction enzymes.   Restriction fragments

may then be separated by electrophoresis on an agarose gel,

before denaturation and transfer to a nitrocellulose filter.

Labelled probe may be hybridised to the DNA fragments on the

20 filter and binding determined.   DNA for probing may be

prepared from RNA preparations from cells by techniques such

as reverse-transcriptase- PRC.

Preliminary experiments may be performed by hybridising under

25 low stringency conditions various probes to Southern blots of

DNA digested with restriction enzymes.   For probing,

preferred conditions are those which are stringent enough for

there to be a simple pattern with a small number of

hybridisations identified as positive which can be
investigated further. It is well known in the art to
increase stringency of hybridisation gradually until only a
few positive clones remain. Suitable conditions would be

5 achieved when a large number of hybridising fragments were
obtained while the background hybridisation was low. Using
these conditions nucleic acid libraries, e.g. cDNA libraries
representative of expressed sequences, may be searched.
Those skilled in the art are well able to employ suitable

10 conditions of the desired stringency for selective
hybridisation, taking into account factors such as
oligonucleotide length and base composition, temperature and
so on.

15 For instance, screening may initially be carried out under
conditions, which comprise a temperature of about 37°C or
more, a formamide concentration of less than about 50%, and a
moderate to low salt (e.g. Standard Saline Citrate ('SSC') =
0.15 M sodium chloride; 0.15 M sodium citrate; pH 7)

20 concentration.

Alternatively, a temperature of about 50°C or more and a high
salt (e.g. 'SSPE'= 0.180 mM sodium chloride; 9 mM disodium
hydrogen phosphate; 9 mM sodium dihydrogen phosphate; 1 mM

25 sodium EDTA; pH 7.4). Preferably the screening is carried out
at about 37°C, a formamide concentration of about 20%, and a
salt concentration of about 5 X SSC, or a temperature of
about 50°C and a salt concentration of about 2 X SSPE. These

conditions will allow the identification of sequences which
have a substantial degree of homology (similarity, identity)
with the probe sequence, without requiring the perfect
homology for the identification of a stable hybrid.

5

Suitable conditions include, e.g. for detection of sequences
that are about 80-90% identical, hybridization overnight at
42°C in 0.25M Na_2HPO_4, pH 7.2, 6.5% SDS, 10% dextran sulfate
and a final wash at 55°C in 0.1X SSC, 0.1% SDS. For

10 detection of sequences that are greater than about 90%
identical, suitable conditions include hybridization
overnight at 65°C in 0.25M Na_2HPO_4, pH 7.2, 6.5% SDS, 10%
dextran sulphate and a final wash at 60°C in 0.1X SSC, 0.1%
SDS.

15

Conditions that may be used to differentiate *Rht* genes and
homologues from others may include the following procedure:

First and second DNA molecules are run on an agarose gel,

20 blotted onto a membrane filter (Sambrook *et al*, 1989). The
filters are incubated in prehybridization solution [6xSSC, 5x
Denhart's solution, 20 mM Tris-HCl, 0.1% SDS, 2mM EDTA, 20
µg/ml Salmon sperm DNA] at 65°C for 5 hours, with constant
shaking. Then, the solution is replaced with 30 ml of the

25 same, containing the radioactively-labelled second DNA
(prepared according to standard techniques; see Sambrook *et
al*, 1989), and incubated overnight at 65°C, with constant
shaking. The following morning the filters are rinsed (one

rinse with 3xSSC-0.1% SDS solution); and then washed: one

wash at 65°C, for 25 minutes, with 3x SSC-0.1% SDS solution;

and a second wash, at the same temperature and for the same

time, with 0.1xSSC-0.1% SDS. Then the radioactive pattern on

5 the filter is recorded using standard techniques (see

Sambrook *et al*, 1989).

If need be, stringencycan be increased by increasing the

temperature of the washes, and/or reducing or even omitting

10 altogether, the SSC ini the wash solution.

(SSC is 150 mM NaCl, 15 mM sodium citrate. 50x Denhart's

solution is 1% (w/v) ficoll, 1% polyvinylpyrrolidone, 1%

(w/v) bovine serum albumin.)

15

Homologues to *rht* mutants are also provided by the present

invention. These may be mutants where the wild-type includes

the 17 amino acids underlined in Figure 3b, or a contiguous

sequence of 17 amino acids with at least about 10 (more

20 preferably 11, 12, 13, 14, 15, 16 or 17) which have

similarity or identity with the corresponding residue in the

17 amino acid sequence underlined in Figure 3, but the mutant

does not. Similarly, such mutants may be where the wild-type

includes DVAQKLEQLE or a contiguous sequence of 10 amino

25 aicds with at least about 5 (more preferably 6, 7, 8 or 9)

which have similarity or identity with the corresponding

residue in the sequence DVAQKLEQLE, but the mutant does not.

Nucleic acid encoding such mutant polypeptides may on

expression in a plant confer a phenotype which is insensitive
or unresponsive to treatment of the plant with GA, that is a
mutant phenotype which is not overcome or there is no
reversion to wild-type phenotype on treatment of the plant
5 with GA (though there may be some response in the plant on
provision or depletion of GA).

A further aspect of the present invention provides a nucleic
acid isolate having a nucleotide sequence encoding a
10 polypeptide which includes an amino acid sequence which is a
mutant, allele, derivative or variant sequence of the Rht
amino acid sequence of the species Triticum Aestivum shown in
Figure 3b, or is a homologue of another species or a mutant,
allele, derivative or variant thereof, wherein said mutant,
15 allele, derivative, variant or homologue differs from the
amino acid sequence shown in Figure 3b by way of insertion,
deletion, addition and/or substitution of one or more amino
acids, as obtainable by producing transgenic plants by
transforming plants which have a Rht null mutant phenotype,
20 which phenotype is resistance to the dwarfing effect of
paclobutrazol, with test nucleic acid, causing or allowing
expression from test nucleic acid within the transgenic
plants, screening the transgenic plants for those exhibiting
complementation of the Rht null mutant phenotype to identify
25 test nucleic acid able to complement the Rht null mutant,
deleting from nucleic acid so identified as being able to
complement the Rht null mutant a nucleotide sequence encoding
the 17 amino acid sequence underlined in Figure 3b or a

contiguous 17 amino acid sequence in which at least 10

residues have similarity or identity with the respective

amino acid in the corresponding position in the 17 amino acid

sequence underlined in Figure 3b, more preferably 11, 12, 13,

5 14, 15, 16 or 17, and/or a nucleotide sequence encoding

DVAQKLEQLE or a contiguous sequence of 10 amino aicds with at

least about 5 (more preferably 6, 7, 8 or 9) which have

similarity or identity with the corresponding residue in the

sequence DVAQKLEQLE.

10

A cell containing nucleic acid of the present invention

represents a further aspect of the invention, particularly a

plant cell, or a bacterial cell.

15 The cell may comprise the nucleic acid encoding the protein

by virtue of introduction into the cell or an ancestor

thereof of the nucleic acid, e.g. by transformation using any

suitable technique available to those skilled in the art.

20 Also according to the invention there is provided a plant

cell having incorporated into its genome nucleic acid as

disclosed.

Where a complete naturally occurring sequence is employed the

25 plant cell may be of a plant other than the natural host of

the sequence.

The present invention also provides a plant comprising such a

plant cell.

Also according to the invention there is provided a plant
cell having incorporated into its genome a sequence of
5 nucleotides as provided by the present invention, under
operative control of a regulatory sequence for control of
expression. A further aspect of the present invention
provides a method of making such a plant cell involving
introduction of a vector comprising the sequence of
10 nucleotides into a plant cell and causing or allowing
recombination between the vector and the plant cell genome to
introduce the sequence of nucleotides into the genome.

A plant according to the present invention may be one which
15 does not breed true in one or more properties. Plant
varieties may be excluded, particularly registrable plant
varieties according to Plant Breeders' Rights. It is noted
that a plant need not be considered a "plant variety" simply
because it contains stably within its genome a transgene,
20 introduced into a cell of the plant or an ancestor thereof.

In addition to a plant, the present invention provides any
clone of such a plant, seed, selfed or hybrid progeny and
descendants, and any part of any of these, such as cuttings,
25 seed. The invention provides any plant propagule, that is
any part which may be used in reproduction or propagation,
sexual or asexual, including cuttings, seed and so on. Also
encompassed by the invention is a plant which is a sexually

or asexually propagated off-spring, clone or descendant of
such a plant, or any part or propagule of said plant, off-
spring, clone or descendant.

5   The invention further provides a method of influencing the
    characteristics of a plant comprising expression of a
    heterologous *Rht* or *rht* gene sequence (or mutant, allele,
    derivative or homologue thereof, as discussed) within cells
    of the plant. The term "heterologous" indicates that the
10 gene/sequence of nucleotides in question have been introduced
    into said cells of the plant, or an ancestor thereof, using
    genetic engineering, that is to say by human intervention,
    which may comprise transformation. The gene may be on an
    extra-genomic vector or incorporated, preferably stably, into
15 the genome. The heterologous gene may replace an endogenous
    equivalent gene, ie one which normally performs the same or a
    similar function in control of growth and/or development, or
    the inserted sequence may be additional to an endogenous
    gene. An advantage of introduction of a heterologous gene is
20 the ability to place expression of the gene under the control
    of a promoter of choice, in order to be able to influence
    gene expression, and therefore growth and/or development of
    the plant according to preference. Furthermore, mutants and
    derivatives of the wild-type gene may be used in place of the
25 endogenous gene. The inserted gene may be foreign or
    exogenous to the host cell, e.g. of another plant species.

The principal characteristic which may be altered using the

present invention is growth.

According to the model of the *Rht* gene as a growth repressor,
under-expression of the gene may be used to promote growth,
5 at least in plants which have only one endogenous gene
conferring *Rht* function (not for example *Arabidopsis* which
has endogenous homologues which would compensate). This may
involve use of anti-sense or sense regulation. Taller plants
may be made by knocking out *Rht* or the relevant homologous
10 gene in the plant of interest. Plants may be made which are
resistant to compounds which inhibit GA biosynthesis, such as
paclobutrazol, for instance to allow use of a GA biosynthesis
inhibitor to keep weeds dwarf but let crop plants grow tall.

15 Over-expression of a *Rht* gene may lead to a dwarf plant which
is correctable by treatment with GA, as predicted by the *Rht*
repression model.

Since *rht* mutant genes are dominant on phenotype, they may be
20 used to make GA-insensitive dwarf plants. This may be
applied for example to any transformable crop-plant, tree or
fruit-tree species. It may provide higher yield/reduced
lodging like Rht wheat. In rice this may provide GA-
insensitive rice resistant to the Bakane disease, which is a
25 problem in Japan and elsewhere. Dwarf ornamentals may be of
value for the horticulture and cut-flower markets. Sequence
manipulation may provide for varying degrees of severity of
dwarfing, GA-insensitive phenotype, allowing tailoring of the

degree of severity to the needs of each crop-plant or the
wishes of the manipulator. Over-expression of *rht*-mutant
sequences is potentially the most useful.

5 A second characteristic that may be altered is plant
development, for instance flowering. In some plants, and in
certain environmental conditions, a GA signal is required for
floral induction. For example, GA-deficient mutant
Arabidopsis plants grown under short day conditions will not
10 flower unless treated with GA: these plants do flower
normally when grown under long day conditions. *Arabidopsis*
gai mutant plants show delayed flowering under short day
conditions: severe mutants may not flower at all. Thus, for
instance by *Rht* or *rht* gene expression or over-expression,
15 plants may be produced which remain vegetative until given GA
treatment to induce flowering. This may be useful in
horticultural contexts or for spinach, lettuce and other
crops where suppression of bolting is desirable.

20 The nucleic acid according to the invention may be placed
under the control of an externally inducible gene promoter to
place the *Rht* or *rht* coding sequence under the control of the
user.

25 The term "inducible" as applied to a promoter is well
understood by those skilled in the art. In essence,
expression under the control of an inducible promoter is
"switched on" or increased in response to an applied

stimulus. The nature of the stimulus varies between

promoters. Some inducible promoters cause little or

undetectable levels of expression (or no expression) in the

absence of the appropriate stimulus. Other inducible

5   promoters cause detectable constitutive expression in the

absence of the stimulus. Whatever the level of expression is

in the absence of the stimulus, expression from any inducible

promoter is increased in the presence of the correct

stimulus. The preferable situation is where the level of

10   expression increases upon application of the relevant

stimulus by an amount effective to alter a phenotypic

characteristic. Thus an inducible (or "switchable") promoter

may be used which causes a basic level of expression in the

absence of the stimulus which level is too low to bring about

15   a desired phenotype (and may in fact be zero). Upon

application of the stimulus, expression is increased (or

switched on) to a level which brings about the desired

phenotype.

20   Suitable promoters include the Cauliflower Mosaic Virus 35S

(CaMV 35S) gene promoter that is expressed at a high level in

virtually all plant tissues (Benfey et al, 1990a and 1990b);

the maize glutathione-S-transferase isoform II (GST-II-27)

gene promoter which is activated in response to application

25   of exogenous safener (WO93/01294, ICI Ltd); the cauliflower

meri 5 promoter that is expressed in the vegetative apical

meristem as well as several well localised positions in the

plant body, eg inner phloem, flower primordia, branching

points in root and shoot (Medford, 1992; Medford et al, 1991) and the *Arabidopsis thaliana LEAFY* promoter that is expressed very early in flower development (Weigel et al, 1992).

5   The GST-II-27 gene promoter has been shown to be induced by certain chemical compounds which can be applied to growing plants. The promoter is functional in both monocotyledons and dicotyledons. It can therefore be used to control gene expression in a variety of genetically modified plants,
10 including field crops such as canola, sunflower, tobacco, sugarbeet, cotton; cereals such as wheat, barley, rice, maize, sorghum; fruit such as tomatoes, mangoes, peaches, apples, pears, strawberries, bananas, and melons; and vegetables such as carrot, lettuce, cabbage and onion. The
15 GST-II-27 promoter is also suitable for use in a variety of tissues, including roots, leaves, stems and reproductive tissues.

Accordingly, the present invention provides in a further
20 aspect a gene construct comprising an inducible promoter operatively linked to a nucleotide sequence provided by the present invention, such as the *Rht* gene of *Triticum* a homologue from another plant species or any mutant, derivative or allele thereof. This enables control of
25 expression of the gene. The invention also provides plants transformed with said gene construct and methods comprising introduction of such a construct into a plant cell and/or induction of expression of a construct within a plant cell,

by application of a suitable stimulus, an effective exogenous
inducer. The promoter may be the GST-II-27 gene promoter or
any other inducible plant promoter.

5   When introducing a chosen gene construct into a cell, certain
considerations must be taken into account, well known to
those skilled in the art. The nucleic acid to be inserted
should be assembled within a construct which contains
effective regulatory elements which will drive transcription.
10 There must be available a method of transporting the
construct into the cell. Once the construct is within the
cell membrane, integration into the endogenous chromosomal
material either will or will not occur. Finally, as far as
plants are concerned the target cell type must be such that
15 cells can be regenerated into whole plants.

Selectable genetic markers may be used consisting of
chimaeric genes that confer selectable phenotypes such as
resistance to antibiotics such as kanamycin, hygromycin,
20 phosphinotricin, chlorsulfuron, methotrexate, gentamycin,
spectinomycin, imidazolinones and glyphosate.

An aspect of the present invention is the use of nucleic acid
according to the invention in the production of a transgenic
25 plant.

A further aspect provides a method including introducing the
nucleic acid into a plant cell and causing or allowing

incorporation of the nucleic acid into the genome of the
cell.

Any appropriate method of plant transformation may be used to

5 generate plant cells comprising nucleic acid in accordance
with the present invention. Following transformation, plants
may be regenerated from transformed plant cells and tissue.

Successfully transformed cells and/or plants, i.e. with the

10 construct incorporated into their genome, may be selected
following introduction of the nucleic acid into plant cells,
optionally followed by regeneration into a plant, e.g. using
one or more marker genes such as antibiotic resistance (see
above).

15

Plants transformed with the DNA segment containing the
sequence may be produced by standard techniques which are
already known for the genetic manipulation of plants. DNA
can be transformed into plant cells using any suitable

20 technology, such as a disarmed Ti-plasmid vector carried by
Agrobacterium exploiting its natural gene transfer ability
(EP-A-270355, EP-A-0116718, NAR 12(22) 8711 - 87215 1984),
particle or microprojectile bombardment (US 5100792, EP-A-
444882, EP-A-434616) microinjection (WO 92/09696, WO

25 94/00583, EP 331083, EP 175966, Green et al. (1987) *Plant
Tissue and Cell Culture*, Academic Press), electroporation (EP
290395, WO 8706614 Gelvin Debeyser - see attached) other
forms of direct DNA uptake (DE 4005152, WO 9012096, US

4684611), liposome mediated DNA uptake (e.g. Freeman *et al.* *Plant Cell Physiol.* 29: 1353 (1984)), or the vortexing method (e.g. Kindle, *PNAS U.S.A.* 87: 1228 (1990d). Physical methods for the transformation of plant cells are reviewed in Oard,

5 1991, *Biotech. Adv.* 9: 1-11.

Agrobacterium transformation is widely used by those skilled in the art to transform dicotyledonous species. Recently, there has been substantial progress towards the routine

10 production of stable, fertile transgenic plants in almost all economically relevant monocot plants (Toriyama, et al. (1988) *Bio/Technology* 6, 1072-1074; Zhang, *et al.* (1988) *Plant Cell Rep.* 7, 379-384; Zhang, *et al.* (1988) *Theor Appl Genet* 76, 835-840; Shimamoto, *et al.* (1989) *Nature* 338, 274-276; Datta,

15 *et al.* (1990) *Bio/Technology* 8, 736-740; Christou, *et al.* (1991) *Bio/Technology* 9, 957-962; Peng, *et al.* (1991) International Rice Research Institute, Manila, Philippines 563-574; Cao, *et al.* (1992) *Plant Cell Rep.* 11, 585-591; Li, *et al.* (1993) *Plant Cell Rep.* 12, 250-255; Rathore, *et al.*

20 (1993) *Plant Molecular Biology* 21, 871-884; Fromm, *et al.* (1990) *Bio/Technology* 8, 833-839; Gordon-Kamm, *et al.* (1990) *Plant Cell* 2, 603-618; D'Halluin, *et al.* (1992) *Plant Cell* 4, 1495-1505; Walters, *et al.* (1992) *Plant Molecular Biology* 18, 189-200; Koziel, *et al.* (1993) *Biotechnology* 11, 194-200;

25 Vasil, I. K. (1994) *Plant Molecular Biology* 25, 925-937; Weeks, *et al.* (1993) *Plant Physiology* 102, 1077-1084; Somers, *et al.* (1992) *Bio/Technology* 10, 1589-1594; WO92/14828). In particular, *Agrobacterium* mediated transformation is now

emerging also as an highly efficient transformation method in monocots (Hiei *et al.* (1994) *The Plant Journal* 6, 271-282).

The generation of fertile transgenic plants has been achieved
5 in the cereals rice, maize, wheat, oat, and barley (reviewed in Shimamoto, K. (1994) *Current Opinion in Biotechnology* 5, 158-162.; Vasil, *et al.* (1992) *Bio/Technology* 10, 667-674; Vain et al., 1995, *Biotechnology Advances* 13 (4): 653-671; Vasil, 1996, *Nature Biotechnology* 14 page 702).
10

Microprojectile bombardment, electroporation and direct DNA uptake are preferred where Agrobacterium is inefficient or ineffective. Alternatively, a combination of different techniques may be employed to enhance the efficiency of the
15 transformation process, eg bombardment with Agrobacterium coated microparticles (EP-A-486234) or microprojectile bombardment to induce wounding followed by co-cultivation with Agrobacterium (EP-A-486233).

20 *Brassica napus* transformation is described in Moloney *et al.* (1989) *Plant Cell Reports* 8: 238-242.

Following transformation, a plant may be regenerated, e.g. from single cells, callus tissue or leaf discs, as is
25 standard in the art. Almost any plant can be entirely regenerated from cells, tissues and organs of the plant. Available techniques are reviewd in Vasil et al., *Cell Culture and Somatic Cel Genetics of Plants, Vol I, II and*

III, Laboratory Procedures and Their Applications, Academic

Press, 1984, and Weissbach and Weissbach, *Methods for Plant*

Molecular Biology, Academic Press, 1989.

5 The particular choice of a transformation technology will be

determined by its efficiency to transform certain plant

species as well as the experience and preference of the

person practising the invention with a particular methodology

of choice. It will be apparent to the skilled person that the

10 particular choice of a transformation system to introduce

nucleic acid into plant cells is not essential to or a

limitation of the invention, nor is the choice of technique

for plant regeneration.

15 In the present invention, over-expression may be achieved by

introduction of the nucleotide sequence in a sense

orientation. Thus, the present invention provides a method

of influencing a characteristic of a plant, the method

comprising causing or allowing expression of nucleic acid

20 according to the invention from that nucleic acid within

cells of the plant.

Under-expression of the gene product polypeptide may be

achieved using anti-sense technology or "sense regulation".

25 The use of anti-sense genes or partial gene sequences to

down-regulate gene expression is now well-established. DNA

is placed under the control of a promoter such that

transcription of the "anti-sense" strand of the DNA yields

RNA which is complementary to normal mRNA transcribed from

the "sense" strand of the target gene. For double-stranded

DNA this is achieved by placing a coding sequence or a

fragment thereof in a "reverse orientation" under the control

5 of a promoter. The complementary anti-sense RNA sequence is

thought then to bind with mRNA to form a duplex, inhibiting

translation of the endogenous mRNA from the target gene into

protein. Whether or not this is the actual mode of action is

still uncertain. However, it is established fact that the

10 technique works. See, for example, Rothstein et al, 1987;

Smith *et al*, (1988) *Nature* 334, 724-726; Zhang *et al*, (1992)

The Plant Cell 4, 1575-1588, English *et al.*, (1996) *The Plant*

Cell 8, 179-188. Antisense technology is also reviewed in

reviewed in Bourque, (1995), *Plant Science* 105, 125-149, and

15 Flavell, (1994) *PNAS USA* 91, 3490-3496.

The complete sequence corresponding to the coding sequence in

reverse orientation need not be used. For example fragments

of sufficient length may be used. It is a routine matter for

20 the person skilled in the art to screen fragments of various

sizes and from various parts of the coding sequence to

optimise the level of anti-sense inhibition. It may be

advantageous to include the initiating methionine ATG codon,

and perhaps one or more nucleotides upstream of the

25 initiating codon. A further possibility is to target a

regulatory sequence of a gene, e.g. a sequence that is

characteristic of one or more genes in one or more pathogens

against which resistance is desired. A suitable fragment

may have at least about 14-23 nucleotides, e.g. about 15, 16

or 17, or more, at least about 25, at least about 30, at

least about 40, at least about 50, or more. Other fragments

may be at least about 300 nucleotides, at least about 400

5 nucleotides, at least about 500 nucleotides, at least about

600 nucleotides, at least about 700 nucleotides or more.

Such fragments in the sense orientation may be used in co-

suppression (see below).

10 Total complementarity of sequence is not essential, though

may be preferred. One or more nucleotides may differ in the

anti-sense construct from the target gene. It may be

preferred for there to be sufficient homology for the

respective anti-sense and sense RNA molecules to hybridise,

15 particularly under the conditions existing in a plant cell.

Thus, the present invention also provides a method of

influencing a characteristic of a plant, the method

comprising causing or allowing anti-sense transcription from

20 nucleic acid according to the invention within cells of the

plant.

When additional copies of the target gene are inserted in

sense, that is the same, orientation as the target gene, a

25 range of phenotypes is produced which includes individuals

where over-expression occurs and some where under-expression

of protein from the target gene occurs. When the inserted

gene is only part of the endogenous gene the number of

under-expressing individuals in the transgenic population

increases. The mechanism by which sense regulation occurs,

particularly down-regulation, is not well-understood.

However, this technique is also well-reported in scientific

5 and patent literature and is used routinely for gene control.

See, for example, See, for example, van der Krol *et al.*,

(1990) *The Plant Cell* 2, 291-299; Napoli *et al.*, (1990) *The*

Plant Cell 2, 279-289; Zhang *et al.*, (1992) *The Plant Cell* 4,

1575-1588, and US-A-5,231,020.

10

Thus, the present invention also provides a method of

influencing a characteristic of a plant, the method

comprising causing or allowing expression from nucleic acid

according to the invention within cells of the plant. This

15 may be used to influence growth.

Aspects and embodiments of the present invention will now be

illustrated, by way of example, with reference to the

accompanying figures. Further aspects and embodiments will

20 be apparent to those skilled in the art. All documents

mentioned in this text are incorporated herein by reference.

The following Figures are included herein:

25 **Figure 1:** Alignment of N-terminus predicted GAI amino acid

sequence (Gai) with rice EST D39460 (0830), with a region of

homology outlined in black.

42

Figure 2:   DNA sequences from C15-1, 14a1 and 5a1.

Figure 2a shows a consensus DNA sequence cDNA C15-1 (obtained
via single-pass sequencing).

5

Figure 2b shows data from original DNA sequencing runs from
14a1 (single-pass).

Figure 2c shows data from original DNA sequencing runs from
10 5a1 (single-pass).

Figure 3:   Rht sequences.

Figure 3a shows a composite DNA sequence of wheat *Rht* gene
15 derived from data in Figure 2, including coding sequence.

Figure 3b shows an alignment of the entire predicted Rht
protein sequence encoded by the coding sequence of Figure 2
(rht) with the entire predicted GAI protein sequence of
20 *Arabidopsis* (Gai).   Regions of sequence identity are
highlighted in black.

Figure 4:   D39460 sequence.

25 Figure 4a shows DNA sequence (single-pass) of rice cDNA
D39460. This cDNA is an incomplete, partial clone, missing
the 3' end of the mRNA from which it is derived.

Figure 4b shows alignment of the entire predicted Rht protein sequence (wheat - encoded by the coding sequence of Figure 2) with that of GAI (Gai) and rice protein sequence predicted from DNA sequence in Figure 4a (Rice). Regions of amino acid

5 identity are highlighted in black; some conservative substitutions are shaded.

Figure 5:   The basic carbon-ring structure of gibberellins.

10 **Figure 6:**   Rice EST sequence

Figure 6a shows the nucleotide sequence of rice EST D39460, as determined by the present inventors.

15 Figure 6b shows the predicted amino acid sequence encoded by the rice EST sequence of Figure 6a.

Figure 7: Wheat C15-1 cDNA

20 Figure 7a shows the nucleotide sequence of the wheat C15-1 cDNA.

Figure 7b shows the predicted amino acid sequence of the wheat C15-1 cDNA of Figure 7a.

25

Figure 8:   Wheat 5a1 genomic clone

Figure 8a shows the nucleotide sequence of the 5a1 wheat

genomic clone.

Figure 8b shows the predicted amino acid sequence of the 5a1
wheat genomic clone of Figure 8a.

5

Figure 9:   Maize 1a1 genomic clone

Figure 9a shows the nucleotide sequence of the 1a1 maize
genomic clone, i.e. D8.

10

Figure 9b shows the amino acid sequence of the maize 1a1
genomic clone of Figure 9a.

Figure 10 shows a PRETTYBOX alignment of amino acid sequences
15 of the maize D8 polypeptide with, the wheat Rht polypeptide
the rice EST sequence determined by the present inventors and
the Arabidopsis thaliana Gai polypeptide.

Figure 11:   Sequences of maize D8 alleles

20

Figure 11a shows a partial nucleotide sequence of the maize
D8-1 allele.

Figure 11b shows a partial amino acid sequence of the maize
25 D8-1 allele.

Figure 11c shows a partial nucleotide sequence of the maize
D8-2023 allele.

Figure 11d shows a partial amino acid sequence of the maize
D8-2023 allele.

Figure 12:   Wheat *rht-10* allele

Figure 12a shows a partial nucleotide sequence of the wheat
rht-10 allele.

Figure 12b shows a partial amino acid sequence of the wheat
rht-10 allele.

Previously, we cloned the *GAI* gene of *Arabidopsis*
(PCT/GB97/00390 - WO97/29123 published 14 August 1997).
Comparison of the DNA sequences of the wild-type (*GAI*) and
mutant (*gai*) alleles showed that *gai* encodes a mutant
predicted protein product (gai) which lacks a segment of 17
amino acids from close to the N-terminus of the protein.
Screening of the DNA sequence databases with the *GAI* sequence
revealed the existence of a rice EST (D39460) which contains
a region of sequence very closely related to that of the
segment that is deleted from GAI in the gai protein. A
comparison of the predicted amino acid sequences from the
region DELLA to EQLE are identical in both sequences. The
two differences (V/A; E/D) are conservative substitutions, in
which one amino acid residue is replaced by another having
very similar chemical properties. In addition, the region of
identity extends beyond the boundary of the deletion region

in the gai protein. The sequence DVAQKLEQLE is not affected

by the deletion in gai, and yet is perfectly conserved

between the GAI and D39460 sequences (Figure 1).

5 An approximately 700 bp *SalI-NotI* subfragment of D39460 was

used in low-stringency hybridization experiments to isolate

hybridizing clones from wheat cDNA and genomic libraries

(made from DNA from the variety Chinese Spring) and from a

maize genomic library (made from line B73N). Several wheat

10 clones were isolated, including C15-1 and C15-10 (cDNAs), and

5a1 and 14a1 (genomic clones). Clone C15-1 has been used in

gene mapping experiments. Nullisomic-tetrasomic analysis

showed that clone C15-1 hybridizes to genomic DNA fragments

derived from wheat chromosomes 4A, 4B and 4D. This is

15 consistent with clone C15-1 containing *Rht* sequence, since

the *Rht* loci map to the group 4 chromosomes. Furthermore,

recombinant analysis using a population segregating for the

Rht-D1b (formerly *Rht2*) allele identified a hybridizing

fragment that displayed perfect co-segregation with the

20 mutant allele. This placed the genomic location of the gene

encoding the mRNA sequence in cDNA C15-1 within a 2 cM

segment (that was already known to contain *Rht*) of the group

4 chromosomes, and provides strong evidence that the cDNA and

genomic clones do indeed contain the *Rht* gene. The maize *D8*

25 DNA sequence disclosed herein is from subcloned contiguous

1.8 kb and 3.0 kb *SalI* fragments (cloned into Bluescript™

SK⁺) from 1a1. The wheat *Rht* sequence disclosed herein is

from a 5.7 kb *DraI* subfragment cloned into Bluescript™ SK⁺)

from clone 5a1.

Figure 2a gives the complete (single-pass) DNA sequence of

cDNA C15-1. We have also obtained DNA sequence for C15-10;

5 it is identical with that of C15-1, and is therefore not

shown. Figures 2b and 2c show original data from individual

sequencing runs from clones 14a1 and 5a1. The sequences

shown in Figure 2 can be overlapped to make a composite DNA

sequence, shown in Figure 3a. This sequence displays strong

10 homology with that of *Arabidopsis GAI*, as revealed by a

comparison of the amino acid sequence of a predicted

translational product of the wheat sequence (Rht) with that

of *GAI* (GAI), shown in Figure 3b. In particular, the

predicted amino acid sequence of the presumptive *Rht* reveals

15 a region of near-identity with GAI over the region that is

missing in gai (Figure 4). Figure 4 reveals that the

homology that extends beyond the gai deletion region in the

rice EST is also conserved in Rht (DVAQKLEQLE), thus

indicating that this region, in addition to that found in the

20 gai deletion, is involved in GA signal-transduction. This

region is not found in SCR, another protein that is related

in sequence to GAI but which is not involved in GA

signalling. The primers used in the above sequencing

experiments are shown in Table 1.

25

Further confirmation that these sequences are indeed the

wheat *Rht* and maize *D8* loci has been obtained by analysis of

gene sequences from various mutant alleles, as follows.

48

The present inventors obtained and sequenced the clone
identified on the database as the rice EST D39460, and the
nucleotide and predicted amino acid sequences resulting from
that work are shown in Figure 6a and Figure 6b respectively.

5

Previous work on the *GAI* gene of *Arabidopsis* showed that the
GAI protein consists of two sections, an N-terminal half
displaying no homology with any protein of known function,
and a C-terminal half displaying extensive homology with the
10 *Arabidopsis* SCR candidate transcription factor (Peng *et al.*
(1997) *Genes and Development* 11: 3194-3205; PCT/GB97/00390).
As described above, deletion of a portion of the N-terminal
half of the protein causes the reduced GA-responses
characteristic of the *gai* mutant allele (Peng et al., 1997;
15 PCT/GB97/00390). The inventors therefore predicted that if
D8 and *Rht* are respectively maize and wheat functional
homologues (orthologues) of *Arabidopsis GAI*, then dominant
mutant alleles of *D8* and *Rht* should also contain mutations
affecting the N-terminal sections of the proteins that they
20 encode.

Previous reports describe a number of dominant mutant alleles
at *D8* and at *Rht*, in particular *D8-1*, *D8-2023* and *Rht-D1c*
(formerly *Rht10*) (Börner *et al.* (1996) *Euphytica* 89: 69-75;
25 Harberd and Freeling (1989) *Genetics* 121: 827-838; Winkler
and Freeling (1994) *Planta* 193: 341-348). The present
inventors therefore cloned the candidate *D8/Rht* genes from
these mutants, and examined by DNA sequencing the portion of

the gene that encodes the N-terminal half of the protein.

A fragment of the candidate *D8* or *Rht* genes that encodes a
portion of the N-terminal half of the D8/Rht protein was
5   amplified via PCR from genomic DNA of plants containing *D8-1*,
D8-2023 and *Rht-D1c*, using the following primers for
amplification: for *D8-1*, primers ZM-15 and ZM-24; for *D8-
2023*, primers ZM-9 and ZM-11; for *Rht-D1c*, nested PCR was
performed using Rht-15 and Rht-26 followed by Rht-16 and Rha-
10 2. PCR reactions were performed using a Perkin Elmer geneAmp
XL PCR kit, using the following conditions: reactions were
incubated at 94°C for 1 min, then subjected to 13 cycles of
94°C, 15 sec - x°C for 15 sec - 69°C 5 min (where x is reduced
by 1°C per cycle starting at 64°C and finishing at 52°C), then
15 25 cycles of 94°C, 15 sec - 53°C, 15 sec - 65°C, 5 min, then
10 min at 70°C. These fragments were then cloned into the
pGEM^R-T Easy vector (Promega, see Technical Manual), and
their DNA sequences were determined.

20 Mutations were found in the candidate *D8* and *Rht* genes in
each of the above mutants. The *D8-1* mutation is an in-frame
deletion which removes amino acids VAQK (55-59) and adds a G
(see sequence in Figure 11a and Figure 11b). This deletion
overlaps with the conserved DVAQKLEQLE homology block
25 described above. *D8-2023* is another in-frame deletion
mutation that removes amino acids LATDTVHYNPSD (87-98) from
the N-terminus of the D8 protein (see Figure 11c and Figure
11d). This deletion does not overlap with the deletion in

gai or D8-1, but covers another region that is highly conserved between GAI, D8 and Rht (see Figure 10). Finally, *Rht-D1c* contains another small in-frame deletion that removes amino acids LNAPPPPLPPAPQ (109-121) in the N-terminal region
5 of the mutant Rht protein that it encodes (see Figure 12a and Figure 12b) (LN-P is conserved netween GAI, D8 and Rht, see Figure 10).

Thus all of the above described mutant alleles are dominant,
10 and confer dwarfism associated with reduced GA-response. All three of these alleles contain deletion mutations which remove a portion of the N-terminal half of the protein that they encode. These observations demonstrate that the *D8* and *Rht* genes of maize and wheat have been cloned.

TABLE 1 - Primers used in the sequencing of Rht

Name	Sequence Sense	
15-L	TTTGCGCCAATTATTGGCCAGAGATAGATAGAGAG	Forward
16-L	GTGGCGGCATGGGTTCGTCCGAGGACAAGATGATG	Forward
23-L	CATGGAGGCGGTGGAGAACTGGGAACGAAGAAGGG	Reverse
26-L	CCCGGCCAGGCGCCATGCCGAGGTGGCAATCAGGG	Reverse
3-L	GGTATCTGCTTCACCAGCGCCTCCGCGGCGGAGAG	Reverse
9-L	ATCGGCCGCAGCGCGTAGATGCTGCTGGAGGAGTC	Reverse
RHA-1	CTGGTGAAGCAGATACCCTTGC	Forward
RHA-2	CTGGTTGGCGGTGAAGTGCG	Reverse
RHA-3	GCAAGGGTATCTGCTTCACCAGC	Reverse
RHA-5	CGCACTTCACCGCCAACCAG	Forward
RHA-6	TTGTGATTTGCCTCCTGTTTCC	Forward
RHA-7	CCGTGCGCCCCCGTGCGGCCCAG	Forward
RHA-8	AGGCTGCCTGACGCTGGGGTTGC	Forward
RHT-9	GATCGGCCGCAGCGCGTAGATGC	Reverse
RHT-10	GATCCCGCACGGAGTCGGCGGACAG	Reverse
RHT-12	TCCGACAGCATGCTCTCGACCCAAG	Reverse
RHT-13	TTCCGTCCGTCTGGCGTGAAGAGG	Forward
RHT-14	AAATCCCGAACCCGCCCCCAGAAC	Forward
RHT-15	GCGCCAATTATTGGCCAGAGATAG	Forward
RHT-16	GGCATGGGTTCGTCCGAGGACAAG	Forward
RHT-18	TTGTCCTCGGACGAACCCATGCCG	Reverse
RHT-19	GATCCAAATCCCGAACCCGCCC	Forward
RHT-20	GTAGATGCTGCTGGAGGAGTCG	Reverse
RHT-21	GTCGTCCATCCACCTCTTCACG	Reverse
RHT-22	GCCAGAGATAGATAGAGAGGCG	Forward
RHT-23	TAGGGCTTAGGAGTTTTACGGG	Reverse
RHT-24	CGGAGTCGGCGGACAGGTCGGC	Reverse
RHT-25	CGGAGAGGTTCTCCTGCTGCACGGC	Reverse
RHT-26	TGTGCAACCCCAGCGTCAGGCAG	Reverse
RHT-27	GCGGCCTCGTCGCCGCCACGCTC	Forward
RHT-28	TGGCGGCGACGAGGCCGCGGTAC	Reverse
RHT-29	AAGAATAAGGAAGAGATGGAGATGGTTG	Reverse
RHT-30	TCTGCAACGTGGTGGCCTGCGAG	Forward
RHT-31	CCCCTCGCAGGCCACCACGTTGC	Reverse
RHT-32	TTGGGTCGAGAGCATGCTGTCGGAG	Forward

52

TABLE 2 - Primers used in the sequence of D-8 clones

	Name	Sequence	Sense
5	ZM-8	GGCGATGACACGGATGACG	Forward
	ZM-9	CTTGCGCATGGCACCGCCCTGCGACGAAG	Reverse
	ZM-10	CCAGCTAATAATGGCTTGCGCGCCTCG	Reverse
	ZM-11	TATCCCAGAACCGAAACCGAG	Forward
	ZM-12	CGGCGTCTTGGTACTCGCGCTTCATG	Reverse
10	ZM-13	TGGGCTCCCGCGCCGAGTCCGTGGAC	Reverse
	ZM-14	CTCCAAGCCTCTTGCGCTGACCGAGATCGAG	Forward
	ZM-15	TCCACAGGCTCACCAGTCACCAACATCAATC	Forward
	ZM-16	ACGGTACTGGAAGTCCACGCGGATGGTGTG	Reverse
	ZM-17	CGCACACCATCCGCGTGGACTTCCAGTAC	Forward
15	ZM-18	CTCGGCCGGCAGATCTGCAACGTGGTG	Forward
	ZM-19	TTGTGACGGTGGACGATGTGGACGCGAGCCTTG	Reverse
	ZM-20	GGACGCTGCGACAAACCGTCCATCGATCCAAC	Forward
	ZM-21	TCCGAAATCATGAAGCGCGAGTACCAAGAC	Forward
	ZM-22	TCGGGTACAAGGTGCGTTCGTCGGATATG	Forward
20	ZM-23	ATGAAGCGCGAGTACCAAGAC	Forward
	ZM-24	GTGTGCCTTGATGCGGTCCAGAAG	Reverse
	ZM-25	AACCACCCCTCCCTGATCACGGAG	Reverse
	ZM-27	CACTAGGAGCTCCGTGGTCGAAGCTG	Forward
	ZM-28	GCTGCGCAAGAAGCCGGTGCAGCTC	Reverse
25	ZM-29	AGTACACTTCCGACATGACTTG	Reverse

<u>CLAIMS</u>:

1.   An isolated polynucleotide encoding a polypeptide which
comprises the amino acid sequence DELLAALGYKVRASDMA and which
on expression in a *Triticum Aestivum* plant provides

5 inhibition of growth of the plant, which inhibition is
antagonised by gibberellin.

2.   An isolated polynucleotide according to claim 1 wherein
the polypeptide includes the amino acid sequence of a *Rht*

10 polypeptide obtainable from *Triticum Aestivum*.

3.   An isolated polynucleotide according to claim 2 which
includes the nucleotide sequence of nucleic acid obtainable
from *Triticum Aestivum* encoding the *Rht* polypeptide, the

15 nucleotide sequence including
GACGAGCTGCTGGCGGCGCTCGGGTACAAGGTGCGCGCCTCCGACATGGCG.

4.   An isolated polynucleotide encoding a polypeptide which
comprises the amino acid sequence shown in Figure 8b.

20

5.   An isolated polynucleotide according to claim 4 which
has the coding nucleotide sequence shown in Figure 8a.

6.   An isolated polynucleotide encoding a polypeptide which

25 on expression in a plant provides inhibition of growth of the
plant, which inhibition is antagonised by gibberellin,
wherein the polypeptide has an amino acid sequence which
shows at least 80% similarity with the amino acid sequence of

the *Rht* polypeptide of *Triticum Aestivum* encoded by nucleic

acid obtainable from *Triticum Aestivum* which includes the

nucleotide sequence

GACGAGCTGCTGGCGGCGCTCGGGTACAAGGTGCGCGCCTCCGACATGGCG.

5

7.   An isolated polynucleotide according to claim 6 wherein

said polypeptide includes the amino acid sequence

DELLAALGYKVRASDMA.

10 8.   An isolated polynucleotide according to claim 6 wherein

said polypeptide includes a contiguous sequence of 17 amino

acids in which at least 10 residues show amino acid

similarity or identity with the residue in the corresponding

position in the amino acid sequence DELLAALGYKVRASDMA.

15

9.   An isolated polynucleotide according to claim 8 wherein

said polypeptide includes a contiguous sequence of 17 amino

acids in which 16 residues show amino acid identity with the

residue in the corresponding position in the amino acid

20 sequence DELLAALGYKVRASDMA.

10. An isolated polynucleotide according to claim 9 wherein

said polypeptide includes the amino acid sequence shown in

Figure 9b for the maize D8 polypeptide.

25

11. An isolated polynucleotide according to claim 10 which

has the coding nucleotide sequence shown in Figure 9a.

12.   An isolated polynucleotide according to claim 9 wherein said polypeptide includes the amino acid sequence shown in Figure 6b.

5 13.   An isolated polynucleotide according to claim 12 which has the coding nucleotide sequence shown in Figure 6a.

14.   An isolated polynucleotide encoding a polypeptide which on expression in a plant confers a phenotype on the plant 10 which is gibberellin-unresponsive dwarfism or which on expression in a *rht* null mutant phenotype plant complements the *rht* null mutant phenotype, such *rht* null mutant phenotype being resistance to the dwarfing effect of paclobutrazol, wherein the polypeptide has an amino acid sequence which 15 shows at least 80% similarity with the amino acid sequence of the *Rht* polypeptide of *Triticum Aestivum* encoded by nucleic acid obtainable from *Triticum Aestivum* which includes the nucleotide sequence GACGAGCTGCTGGCGGCGCTCGGGTACAAGGTGCGCGCCTCCGACATGGCG.

20

15.   An isolated polynucleotide according to claim 14 wherein the polypeptide includes the amino acid sequence of a *Rht* polypeptide obtainable from *Triticum Aestivum*, with one or more amino acids deleted.

25

16.   An isolated polynucleotide according to claim 15 wherein the amino acid sequence DELLAALGYKVRASDMA is deleted.

56

17. An isolated polynucleotide according to claim 15 wherein the amino acid sequence LNAPPPPLPPAPQ is deleted.

18. An isolated polynucleotide according to claim 14 wherein
5   the polypeptide includes the amino acid sequence shown in Figure 9b for the maize D8 polypeptide, with one or more amino acids deleted.

19. An isolated polynucleotide according to claim 18 wherein
10 the amino acid sequence DELLAALGYKVRSSDMA is deleted.

20. An isolated polynucleotide according to claim 19 which has the coding nucleotide sequence shown in Figure 9a, wherein the nucleotides encoding the amino acid sequence
15 DELLAALGYKVRSSDMA are deleted.

21. An isolated polynucleotide according to claim 18 wherein the amino acid sequence VAQK is deleted.

20 22. An isolated polynucleotide according to claim 18 wherein the amino acid sequence LATDTVHYNPSD is deleted.

23. An isolated polynucleotide according to claim 14 wherein the polypeptide includes the amino acid sequence shown in
25 Figure 6b, with one or more amino acids deleted.

24. An isolated polynucleotide according to claim 23 wherein the amino acid sequence DELLAALGYKVRSSDMA deleted.

25.   An isolated polynucleotide according to claim 24 which
has the coding nucleotide sequence shown in Figure 6a,
wherein the nucleotides encoding the amino acid sequence
DELLAALGYKVRSSDMA are deleted.

5

26.   An isolated polynucleotide encoding a polypeptide which
comprises the amino acid sequence shown in Figure 8b, with
the amino acid sequence DELLAALGYKVRASDMA deleted.

10 27.   An isolated polynucleotide according to claim 26 which
has the coding nucleotide sequence shown in Figure 8a,
wherein the nucleotides encoding the amino acid sequence
DELLAALGYKVRASDMA are deleted.

15 28.   An isolated polynucleotide wherein a polynucleotide
according to any of claims 1 to 27 is operably linked to a
regulatory sequence for expression.

29.   An isolated polynucleotide according to claim 28 wherein
20 the regulatory sequence includes an inducible promoter.

30.   An isolated polynucleotide of which the nucleotide
sequence is complementary to a sequence of at least 50
contiguous nucleotides of the coding sequence or sequence
25 complementary to the coding sequence of nucleic acid
according to any of claims 1 to 27 suitable for use in anti-
sense or sense regulation ("co-suppression") of expression
said coding sequence and under control of a regulatory

sequence for transcription.

31. A polynucleotide according to claim 30 wherein the regulatory sequence includes an inducible promoter.

5

32. A nucleic acid vector suitable for transformation of a plant cell and including a polynucleotide according to any preceding claim.

10 33. A host cell containing a heterologous polynucleotide or nucleic acid vector according to any preceding claim.

34. A host cell according to claim 33 which is microbial.

15 35. A host cell according to claim 33 which is a plant cell.

36. A plant cell according to claim 35 having heterologous said polynucleotide within its chromosome.

20 37. A plant cell according to claim 36 having more than one said polynucleotide per haploid genome.

38. A plant cell according to any of claims 35 to 37 which is comprised in a plant, a plant part or a plant propagule, 25 or an extract or derivative of a plant.

39. A method of producing a cell according to any of claims 33 to 37, the method including incorporating said

polynucleotide or nucleic acid vector into the cell by means of transformation.

40. A method according to claim 39 which includes recombining the polynucleotide with the cell genome nucleic acid such that it is stably incorporated therein.

41. A method according to claim 39 or claim 40 which includes regenerating a plant from one or more transformed cells.

42. A plant comprising a plant cell according to any of claims 35 to 37.

43. A part or propagule of a plant comprising a plant cell according to any of claims 35 to 37.

44. A method of producing a plant, the method including incorporating a polynucleotide or nucleic acid vector according to any of claims 1 to 32 into a plant cell and regenerating a plant from said plant cell.

45. A method according to claim 44 including sexually or asexually propagating or growing off-spring or a descendant of the plant regenerated from said plant cell.

46. A method of influencing a characteristic of a plant, the method including causing or allowing expression from a

60

heterologous polynucleotide according to any of claims 1 to
31 within cells of the plant.

47.   Use of a polynucleotide according to any of claims 1 to
5 32 in the production of a transgenic plant.

48.   A method of identifying or obtaining a polynucleotide
according to claim 6, the method including screening
candidate nucleic acid using a nucleic acid molecule which
10 specifically hybridises with a polynucleotide according to
any of claims 1 to 13.

49.   A method according to claim 48 wherein oligonucleotide
primers are employed in PCR.
15

50.   A method according to claim 49 wherein said primers are
selected from those shown in Tables 1 and 2.

51.   An isolated polypeptide encoded by a polynucleotide
20 according to any of claims 1 to 27.

52.   An antibody including an antigen-binding site with
specific binding affinity for the polypeptide according to
claim 51.
25

53.   A polypeptide including the antigen-binding site of an
antibody according to claim 52.

54. A method of identifying or obtaining a polypeptide according to claim 51, the method including screening candidate polypeptides with an antibody or polypeptide according to claim 52 or claim 53.

Figure 1

Gai   . . . . . M K R D   H H H H H Q D K T   M M N E E D D G N   G M DELLAVLG   YKVRSSEMAD   VAQKLEQLEV   54
0803 E A G G S S G G G S   S A D M G S C R D K   V M A G A x G E E E   x V DELLAALG   YKVRSSDMAD   VAQKLEQLEM   60

Gai   M x S N V Q E D D L   S x L A x E T V H Y   N x P A E L Y T W L D . .
0803 A x G M G G V T P P   A x O R M x G S C R T   W x P R T K F I . .

Figure 2a

CCCCGACGGTCGCGGCCGCGGCCAACGCGACGCCCGCGCTGCCGGTCGTCGTGG
TCGACACGCAGGAGGCCGGGATTCGGCTGGTGCACGCGCTGCTGGCGTGCGCGG
AGGCCGTGCAGCAGGAGAACCTCTCCGCCGCGGAGGCGCTGGTGAAGCAGATAC
CCTTGCTGGCCGCGTCCCAGGGCGGCGCGATGCGCAAGGTCGCCGCCTACTTCGG
CGAGGCCCTCGCCCGCCGCGTCTTCCGCTTCCGCCCGCAGCCGGACAGCTCCCTC
CTCGACGCCGCCTTCGCCGACCTCCTCCACGCGCACTTCTACGAGTCCTGCCCCTA
CCTCAAGTTCGCGCACTTCACCGCCAACCAGGCCATCCTGGAGGCGTTCGCCGGC
TGCCGCCGCGTGCACGTCGTCGACTTCGGCATCAAGCAGGGGATGCAGTGGCCC
GCACTTCTCCAGGCCCTCGCCCTCCGTCCCGGCGGCCCTCCCTCGTTCCGCCTCAC
CGGCGTCGGCCCCCGCAGCCGGACGAGACCGACGCCCTGCAGCAGGTGGGCTG
GAAGCTCGCCCAGTTCGCGCACACCATCCGCGTCGACTTCCAGTACCGCGGCC
TCGTCGCCGCCACGCTCGCGGACCTGGAGCCGTTCATGCTGCAGCCGGAGGGCG
AGGAGGACCCGAACGAAGANCCCGANGTAATCGCCGTCAACTCAGTCTTCGAGA
TGCACCGGCTGCTCGCGCAGCCCGGCGCCCTGGAAAAGGTTCTTGGGCACCGTGC
GCCCCCGTGCGGCCCAGAATTCNTCACCGTGGTGGAAACAGGAGGCAAATCACA
ACTCCGGCACATTCCTGGACCGCTTCACCGAGTCTCTGCACTACTACTCCACCAT
GTTCGATTCCCTCGAGGGCGGCAGCTCCGGCGGCGGCCCATCCGAAGTCTCATCG
GGGGCTGCTGCTGCTCCTGCCGCCGCCGGCACGGACCAGGTCATNTCCGAGGTGT
ACCTCGGCCGGCAGATCTGCAACGTGGTGGCCTGCGAGGGGGCGGAACGCACAG
ANCGCCACGAGACGCTGGGCCAGTGGCGGAACCGGCTGGGCAACGCCGGGTTCG
AGACCGTCCACCTGGGCTCCAATGCCTACAAGCAGGCGANCACGCTGCTGGCGC
TCTTCGCCGGCGGCGAACGGCTACANGTGGAAGAAAAGGAAGGCTGCCTGACGC
TGGGGTTGCACACNCCCCCCTGATTGCCACCTCGGCATGGCGCCTGGCCGGGCCG
TGATCTCGCGAGTTTTGAACGCTGTAAGTACACATCGTGAGCATGGAGGACAACA
CAGCCCCGGCGGCCGCCCCGGCTCTCCGGCGAACGCACGCACGCACGCACTTGA
AGAAGAAGAAGCTAAATGTCATGTCAGTGAGCGCTGAATTGCAGCGACCGGCTA
CGATCGATCGGGCTACGGGTGGTTCCGTCCGTCTGGCGTGAAGAGGTGGATGGA
CGACGAACTCCGAGCCGACCACCACCGGCATGTAGTAATGTAATCCCTTCTTCGT
TCCCAGTTCTCCACCGCCTCCATGATCACCCGTAAAACTCCTAAGCCCTATTATTA
CTACTATTATGTTTAAATGTCTATTATTGCTATGTGTAATTCCTCCAACCGCTCAT
ATCAAAATAAGCACGGGCCGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAA

Figure 2b(1)

CGCGCAATGCTTAAGGTCNCCGCCTACTTCGGNGCAGGCCCTCGCCCGCCGCGTC
TTCCGCTTCCGCCCGCAGCCGGACAGCTCCCTCCTCGACGCCGCCTTCGCCGACCT
CCTCCACGCGCACTTCTACNAGTCCTGCCCCTACCTCAAGTTCGCGCACTTCACCG
CCAATTAGGCCATCCTGGAGGCGTTCGCCGGCTGCCGCCGCGTGCACGTCGTCGA
CTTCGGCATCAAGCAGGGGATGCAGTGGCCCGCACTTCTCCAGGCCCTCGCCCTC
CGTCCCGGCGGCCCTCCCTCGTTCCGCCTCACCGGCGTCGGCCCCCGCAGCCGG

Figure 2b(2)

ACCTCCTTCGTCGTCTNTNNGGTGGGGGCGCCAGGAGCTTATGTGGTGGAGGNTG
GCCCCNCCGGTCGCGACCGCGNCCTACGNACGCCCGCGCTGCCGGTCGTCGTGG
TCGACACGCAGGAGGCCGGGATTCGGNTGGTNCACGCGCTGCTGGNGTGCGNGG
AGNCCGTGCAGCAGGAGAACCTCTCCGCCGCGGAGGCGCTNGTGAAGNAGATAC
CCNTGCTGGCCGAGTCCCAGGGCGGCGAGATGNGCAAGGTNGCAGCTTACTTNG
NAGANGCCCTCGCCCGCNGAGTGATTCCACTTANCGCCTGCAGCCGGANAGCTCC
GTCCTCGAANCCGCNTTNGCCGACCTCCTCCACGNGCACNTNTACGAGTC

Figure 2b(3)

TANTAGTCTCTCGGTGGGGGCGCCAGGAGCTCTNTGGTGGAGGCNGCCCCGCCG
GTCGCGGCCGCGGCCAACGCGACGCCCGCGCTGCCGGTCGTCGTGGTCGACACG
CAGGAGGCCGGGATTCGGATGGTGCACGCGCTGNTGGCGTGCGCGGAGGCCGTG
AAACAGTTGAAGGNCCNCGCCTNNNNNCNCACAANNTGAAAGCCCCGNG

Figure 2b(4)

GGCTNCCNCCNCGTGCACGTCGTCGACTTCGGCATCAAGCATGGGATGCANTGGC
NCGNACTTCTCCANGCCCTCGCCCTCCGTCCCGGCGGCCCTCCCTCGTTCCGCCTC
ACCGGCGTCGGCCCCCGCAGCCGGACGAGACCGACGCCCTGCANCAGGTGGGC
TGGAAGCTCGCCCAGTTCGCGCACACCATCCGCGTCGACTTCCANTACCGTGGCC
TCGTCGCCGCCACGCTCGCGGACCTGGAGCCGTTCATGCTGCANCCGGAGGGCGA
GGAGGACCCGAACGACGGAGCCCGAGGTAATCGCCGTCAACTCAGTCTTCGAGA
TGCACCGGGCTGCTCNCGCANCCCGGCGACNCTGGAANAA

Figure 2b Continued

Figure 2b(5)

CAAGANGCTAATCACAACTCCGGCACATTCCTGGACCGCTTCACCGAGTCTCTGC
ANTACTACTCCACCATGTTCGATTCCCTCGAGGGCGGCAGCTCCGGCGGCGGCCC
ATCCGAAGTCTCATCGGGGGCTGCTGCTGCTCCTGCCGCCGCCGGCACGGACCAT
GTCATGTCCGAXGTGTACCTCGGCCGGCAGATCTGCAACGTGGTGGCCTGCGAGG
GGGCGGAGCGCACANTANCGCCACGCAGACNCTGGGCCAGTGGCGTGAACCGGC
TGGGCAACGCCNGGTTCANNNNCCGTCCACCTGGGCTCCAATGCCTACAATCAN
GCNNNCACGCTGCTGGCGCCTCTTCGCCC

Figure 2b(6)

TCGCCANTCGGCATGGNGCCTGGCCGGGCCGTGATCTCGCGAGTTTTGAACGCTG
TAAGTACACATCGTGAGCATGGAGGACAACACAGCCCCGGCGGCCGCCCCGGCT
CTCCGGCGAACGCACGCACGCACGCACTTGGAAGAAGAANAAGCTAAATGTCAT
GTCAGTGAGCGCTGAATTGCAACGACCGGCTACGATCGATCGGGCTACGGGTGG
TTCCGTCCGTCTGGCGTGAAGAGGTGGATGGACGACGAACTCCGANCCGACCAC
CACCGGCATGTAGTAATGTAATCCCTTCTTCGTTCCCAGTTCTCCACCGCCTCCAT
GGATCACCCGTAAAACTCCTAAGCCCTAATTATNNACTAACTAATTATGTTTTAA
AATGTTCTAATTAATTGGCTATGTTGTAATNCCTCCAAACCGGCTCATTTTCAAA
NATTAAGCCACGGGCCCGGAACTTTGGTTTAACAACCTCCCNATTGNAAAATTNA
AATNGAAATTTTTGGTTNC

Figure 2b(7)

GTTGGTGGNGGCGATTTGGGTACAAGGTGCGCGCCTCCGACATGGNGGANGTGG
GGCAGAAGCTGGAGCAGNTCGAGATGGCCATGGGGATGGGNGGCGTGGGCGCT
GGCGCCGCCCCTGACGACAGGTTNGCCACCCGCNGGCCGCGGACACNGTGCANT
ACAACCCCACNGACNTGTCGTCTTGGGTCGAGAGCATGCTGTCGGAGCTAAANG
AGCCGCNGCCGCCCCTCCCGCCCGCCCCGCAGCTCAACGCCTCCACCTCCTCCAC
CGTCACGGGCAGCGGCGGCTACTTCGATAACCCTCCCTG

Figure 2b(8)

TGATGGNGGGAGNTTANGGGGTTANAAATGTGGGGGANTTCCGAANNGGTGAGG
ANATATNNTCAGAAGTTGGAGCAGATGAGAGATNGCTGATGGGGATAGGGTAGG
NGTGGGTGCCGGTGCNGCCCCCNAGGANAGATTGGCCACCCACTTAGCAAGTGG
ANACCGTGGATTACNACCCCACAGACCTGTCGTGGTTGGGTTTGAGAGCGTGGTG
TGGGAGCTGAACGGGCNGCGGCGTGCCCCTCCCGCCCGCCCCGCAGCTCAACGCC
TCCACCTCCTCCACCGTACACGGGCAGCGGCGGCTAGTTCGATCTCCCGCCCTCC
GTCGACTCCTCCAGCAGCATNTANGCGCTGCGGCCGATCCCCTNCCCAAGCNNGC
GNGGNCCGAGCCGTGTAN

Figure 2b Continued

Figure 2b(9)

TTTCANTTTCNTCCTTTTTTCTTCTTTTTCCAACCCCCGGCCCCCNGACCCTTGGAT
CCAAATCCCGAACCCGCCCCCAGAACCNGGAACCGAGGCCAAGCAAAAGNTTTG
CGCCAATTATTGGCCAGAGATAGATAGAGAGGCGAGGTAGCTCGCGGATCATGA
AGCGGGAGTACCAGGACGCCGGAGGGAGCGGCGGCGGCGGTGGCGGCATGGGT
TCGTCCGAGGACAAGATGATGGTGTCGGCGGCGGCGGGGGAGGGGGAGGAGGT
GGACGAGCTGCTGGCGGCGCTCGGGTACAAGGTGCGCGCCTCCGACATGGCGGA
CGTGGCGCAGAAGCTGGAGCAGCTCGAGATGGCCATGGGGATGGGCGGCGTGGG
CGCCGGCGCCGCCCCGACGACAGCTTCGCCACCCACCTCGCCACGGACACCGTG
CAGTACAACCNCCCNGACC

Figure 2b(10)

GGACGACGACCTCCGAGCCGACCACCACCGGCATGTAGTAATGTAATCCCTTCTT
CNTTCCCAGTNCTCCACCGCCTCCATGATCACCCGTAAAACTCCTAAGCCCTATT
ATTACTACTATTATGTNTAANTGTCTATTATTGCTANGTGTAATTCCTCCAACCGC
TCATATCAAAATAAGCACGGGCCGGACTTTGTTANCAGCTCCAATGAGAATGAA
ATGAATTTTGTACGCAAGGCACGTCCAAAACTGGGCTGAGCTTTGTTCTGTTCTG
TTATGTTCATGGTGCTCACTGCTCTGATGAACATGATGGTGCCTCCAATGGTGGC
TTTGCAATTGTTGAAACGTTTGGCTTGGGGGACTTGNGTGGGTGGGTGCATGGGG
ATGAATATTCACATCNCCGGATTAAAATTAAGCCATCCCGTTGGCCGTCCTTTGA
ATANCTTGCCCNAAACGAAATTTCCCCCNATC

Figure 2b(11)

AAANCCTANAANATATAGAGGCGATGTNGCNCCCCNATCANNAACNGGATTACN
GNAACNCCNGAAGGAGCGGCGGCGGCGGTGGCAGCATNGGCTCGTCCGATGACA
AATATCATGGTGTCGGCGGCGGCGGGGGACGGGGAGGAGGTGCACAACNTTTNG
GCGGGACTCGNGTACCACGTGNACGGTGCCGCNCTNGNGGATNTGGCCCTNGAA
GATGGGCCACCTCCAAA

Figure 2b Continued

Figure 2b(12)

CGGCGGCCCCGTGGCGGCATGGGCTCGTCCGAGGACNAGATGATGGTGTCGGCG
GCGGCGGGGGANGGGGATGATGTGGACTATCTGCTGGCGGCGCTCGGGTACAAG
GTGCGCGCCTCCGACAGGCGGAGCCCGCGCATAACTGGAGCCGCTCGAGATGGC
CNTGGGGATNGGCGGCNTGGGCNCCNGCGCCTCCCCCG

Figure 2b(13)

TGGNGCTCGGGTGNCCCGTGCGCGCCTCCGACATGGCGGGACGTGGCGCAGAAC
TGGAGCAGCTCGAGATGGCCATGGGGATGGGCGGCGTGGGCGCCGGCGCCGCCC
CCGACGACAGCTTCGCCACCCACCTCGCCACGGACACCGGCACACAACCCCACCG
ACCTGTCGTCTTGGGTCGAGAGCATGCTGTCGGATCTCNACGCGCCNCCGNCGCC
CCTCCCGCCCGC

Figure 2c(1)

ANNTTGTNCNNNNTACATCCCATGNGCCGCGCNATGCTNAAGGTCGCCGCCTACT
TCGGCGCAGGCCCTCGCCCGCCGCGTCTTCCGCTTCCGCCCGCAGCCGGACAGCT
CCCTCCTCGACGCCGCCTTCGCCGACCTCCTCCACGCGCACTTCTACGAGTCCTGC
CCCTACCTCAAGTTCGCGCACTTCACCGCCAACCAGGCCATCCTGGAGGCGTTCG
CCGGCTGCCGCCGCGTGCACGTCGTCGACTTCGGCATCAAGCAGGGGATGCAGT
GGCCCGCACTTCTCCAGGCCCTCGCCCTCCGTCCCGGCGGCCCTCCCTCGTTCCGC
CTCACCGGCGTTCGGCCCCCGCAGCCGGACGANAACGACGCCCTG

Figure 2c(2)

NTTCCCCGGCAGTTAAAAGCNTCCACTTCTTCCACCGTCACGGGCAGCGGCGGNT
ACTTNGATCTCCCGCCCTCAGTCGACTCCTCCAGCAGCATCTACGCGCTGCGGCC
GATCCCCTCCCCGGCCGGCGCGACGGCGCCGGCCGACCTGTCCGCCGACTCCGTG
CGGGATCCCAAGCGGATGCGCACTGGCGGGAGCAGCACCTCGTCGTCATCCTCCT
CATANTCGTCTCTCGGTGGGGGCGCCAGGAGCTCTGTGGTGGAGGCNGCCCCGCC
GGTCGCGGCCGCGGCCAACGCGACGCCCGCGCTGCCGGTCGTCGTGGTCGACAC
GCAGGAGGCCGGGATTCGGATGGTGCACGCGCTGNTGGCGTGCGCGGAGGCCGT
GNAAGCAGTTNGAAGGGCCTNCGCCGTGNATNNCGCAACAANNNGGAAGNCCN

Figure 2c(3)

CANCCCGCTGNTCGCCACCTCGGCATGGCGCCTGGCCGGGCCGTGATCTCGCGAG
TTTTGAACGCTGTAAGTACACATCGTGAGCATGGAGGACAACACAGCCCCGGCG
GCCGCCCCGGCTCTCCGGCGAACGCACGCACGCACGCACTTGAAGAAGAAGAAG
CTAAATGTCATGTCAGTGAGCGCTGAATTGCANCGACCGGCTACGATCGATCGG
GCTACGGGTGGTTCCGTCCGTCTGGCGTGAAGAGGTGGATGGACGACGAACTCC
GANCCGACCACCACCGGCATGTAGTAATGTAATCCCTTCTTCGTTCCCAGTTTCTC
CACCGCCTCCATGATCACCCCGTAAAACTCCTAAGCCCTATNNNTTACTACNATT
AATGTTTTAAANTGTTCTANTAATTGCTATGNTGTTTATTNCC

Figure 2c(4)

TATCGAAGTAGCCGCCGCTGCCCNTGCACGGTGGAGGAGGTGGAGGCGTTGAGC
TGCGGGGCGGGCGGGAGGGGCGGCGGCGGCACGTTNAGCTCCGACAGCATGCTC
TCGACCCAAAACNACAGGTCGGTGGGGTTGTAGTGCACGGTGTCCGTGGCGAGG
GGGTGGCNAANCTGTCGTCAGGGGCGGCGCCNGCGCCCACNCCGCCCATCCCCA
TGGCCATCTCGANCTGCTCCAGCTTCTGCGCCACTTCCNCCATGTCNGATGCGCG
CNCCTTGTACCCGA

Figure 2c Continued

Figure 2c(5)

ACGGCGCGGNCCNCGCNNGCTTGGGAGGGGATCGGCCGCAGCGCNTANATGCTG
CTGGAGGAGTCGACGGAGGGCGGGAGATCGAACTAGCCGCCGCTGCCCGTGTAC
GGTGGAGGAGGTGGAGGCGTTGAGCTGCGGGGCGGGCGGGAGGGGCAGCNGCT
GCACGTTNAGCTCCCACACCACGTCTCTCAACCCAACCACGACNCGTCTGTGGGG
TNGTAATNCACGGTNTCCCTNGCTANGTGGGTGGCCAATCTNT

Figure 2c(6)

CACGGTGTCCGTGGCGAGGTGGGTGGCGAAGCTGTCGTCGGGGGCGGCGCCGGC
GCCCACGCCGCCCATCCCCATGGCCATCTCGAGCTGCTCCAGCTTCTGCGCCACG
TCCGCCATGTCGGAGGCGCGCACCTTGTACCCGAGCGCCGCCAGCAGCNCGNCC
ACCTCCTCCCCCTCCCCCGCCGCCGCCGACACCATCATCTTGTCCTCGGACGANCC
CATGCCGCCACCGCCGCCGCCGCTCCCTCCGGCGTCCTGGTACTCCCGCTTCATG
ATCCGCGAGCTACCTCGCCTCTCTATCTATCTCTGGCCAATAATTGCGCA

Figure 2c(7)

GACCACCACCGGCATGTAGTAATGTAATCCCTTCTTCNTTCCCAGTTCTCCACCGC
CTCCATGATCACCCGTAAAACTCCTAAGCCCTATTATTACTACTATTATGTNTAA
ATGTCTATTATTGCTANGTGTAATTCCTCCAACCGCTCATATCAAAATAAGCACG
GGCCGGACTTTGTTAGCAGCTCCAATGAGAATGAAATGAATTTTGTACGCAAGGC
ACGTCCAAAACTGGGCTGAGCTTTGTTCTGTTCTGTTATGTTCATGGTGCTCACTG
CTCTGATGAACATGATGGTGCCTCCAATGGGTGGCTTTGCAATTGTTGAACGTTT
TGGCTTGGGGGACTTGGTGNNTGGTGCATGGGAATGAANATTCCACATCCNCNG
GAATTAAAATTAGCCCATCCCG

Figure 3a

TTTCANTTTCNTCCTTTTTTCTTCTTTTTCCAACCCCCGGCCCCCNGACCCTTGGATCC
AAATCCCGAACCCGCCCCCAGAACCNGGAACCGAGGCCAAGCAAAAGNTTTGCGCC
AATTATTGGCCAGAGATAGATAGAGAGGCGAGGTAGCTCGCGGATCATGAAGCGGG
AGTACCAGGACGCCGGAGGGAGCGGCGGCGGCGGTGGCGGCATGGGTTCGTCCGAG
GACAAGATGATGGTGTCGGCGGCGGCGGGGGAGGGGGAGGAGGTGGACGAGCTGC
TGGCGGCGCTCGGGTACAAGGTGCGCGCCTCCGACATGGCGGACGTGGCGCAGAAG
CTGGAGCAGCTCGAGATGGCCATGGGGATGGGCGGCGTGGGCGCTGGCGCCGCCCC
TGACGACAGGTTNGCCACCCGCNGGCCGCGGACACNGTGCANTACAACCCCACNGA
CNTGTCGTCTTGGGTCGAGAGCATGCTGTCGGAGCTAAANGAGCCGCNGCCGCCCC
TCCCGCCCGCCCCGCAGCTCAACGCCTCCACCGTCACGGGCAGCGGCGGNTACTTNG
ATCTCCCGCCCTCAGTCGACTCCTCCAGCAGCATCTACGCGCTGCGGCCGATCCCCT
CCCCGGCCGGCGCGACGGCGCCGGCCGACCTGTCCGCCGACTCCGTGCGGGATCCC
AAGCGGATGCGCACTGGCGGGAGCAGCACCTCGTCGTCATCCTCCTCATANTCGTCT
CTCGGTGGGGGCGCCAGGAGCTCTGTGGTGGAGGCNGCCCCGCCGGTCGCGGCCGC
GGCCAACGCGACGCCCGCGCTGCCGGTCGTCGTGGTCGACACGCAGGAGGCCGGGA
TTCGGCTGGTGCACGCGCTGCTGGCGTGCGCGGAGGCCGTGCAGCAGGAGAACCTC
TCCGCCGCGGAGGCGCTGGTGAAGCAGATACCCTTGCTGGCCGCGTCCCAGGGCGG
CGCGATGCGCAAGGTCGCCGCCTACTTCGGCGAGGCCCTCGCCCGCCGCGTCTTCCG
CTTCCGCCCGCAGCCGGACAGCTCCCTCCTCGACGCCGCCTTCGCCGACCTCCTCCA
CGCGCACTTCTACGAGTCCTGCCCCTACCTCAAGTTCGCGCACTTCACCGCCAACCA
GGCCATCCTGGAGGCGTTCGCCGGCTGCCGCCGCGTGCACGTCGTCGACTTCGGCAT
CAAGCAGGGGATGCAGTGGCCCGCACTTCTCCAGGCCCTCGCCCTCCGTCCCGGCGG
CCCTCCCTCGTTCCGCCTCACCGGCGTCGGCCCCCCGCAGCCGGACGAGACCGACGC
CCTGCAGCAGGTGGGCTGGAAGCTCGCCCAGTTCGCGCACACCATCCGCGTCGACTT
CCAGTACCGCGGCCTCGTCGCCGCCACGCTCGCGGACCTGGAGCCGTTCATGCTGCA
GCCGGAGGGCGAGGAGGACCCGAACGAAGANCCCGANGTAATCGCCGTCAACTCA
GTCTTCGAGATGCACCGGCTGCTCGCGCAGCCCGGCGCCCTGGAAAAGGTTCTTGGG
CACCGTGCGCCCCGTGCGGCCCAGAATTCNTCACCGTGGTGGAAACAGGAGGCAA
ATCACAACTCCGGCACATTCCTGGACCGCTTCACCGAGTCTCTGCACTACTACTCCA
CCATGTTCGATTCCCTCGAGGGCGGCAGCTCCGGCGGCGGCCCATCCGAAGTCTCAT
CGGGGGCTGCTGCTGCTCCTGCCGCCGCCGGCACGGACCAGGTCATNTCCGAGGTGT
ACCTCGGCCGGCAGATCTGCAACGTGGTGGCCTGCGAGGGGGCGGAACGCACAGAN
CGCCACGAGACGCTGGGCCAGTGGCGGAACCGGCTGGGCAACGCCGGGTTCGAGAC
CGTCCACCTGGGCTCCAATGCCTACAAGCAGGCGANCACGCTGCTGGCGCTCTTCGC
CGGCGGCGAACGGCTACANGTGGAAGAAAAGGAAGGCTGCCTGACGCTGGGGTTGC
ACACNCCCCCCTGATTGCCACCTCGGCATGGCGCCTGGCCGGGCCGTGATCTCGCGA
GTTTTGAACGCTGTAAGTACACATCGTGAGCATGGAGGACAACACAGCCCCGGCGG
CCGCCCCGGCTCTCCGGCGAACGCACGCACGCACGCACTTGAAGAAGAAGAAGCTA
AATGTCATGTCAGTGAGCGCTGAATTGCAGCGACCGGCTACGATCGATCGGGCTAC
GGGTGGTTCCGTCCGTCTGGCGTGAAGAGGTGGATGGACGACGAACTCCGAGCCGA
CCACCACCGGCATGTAGTAATGTAATCCCTTCTTCGTTCCCAGTTCTCCACCGCCTCC
ATGATCACCCGTAAAACTCCTAAGCCCTATTATTACTACTATTATGTTTAAATGTCTA
TTATTGCTATGTGTAATTCCTCCAACCGCTCATATCAAAATAAGCACGGGCCGGACT
TTGTTANCAGCTCCAATGAGAATGAAATGAATTTTGTACGCAAGGCACGTCCAAAA
CTGGGCTGAGCTTTGTTCTGTTCTGTTATGTTCATGGTGCTCACTGCTCTGATGAACA
TGATGGTGCCTCCAATGGTGGCTTTGCAATTGTTGAAACGTTTGGCTTGGGGGACTT
GNGTGGGTGGGTGCATGGGGATGAATATTCACATCNCCGGATTAAAATTAAGCCAT
CCCGTTGGCCGTCCTTTGAATANCTTGCCCNAAACGAAATTTCCCCCNATC

Figure 3b

PRETTYBOX of: My.Msf(*) August 7, 1997 13:06:42.76
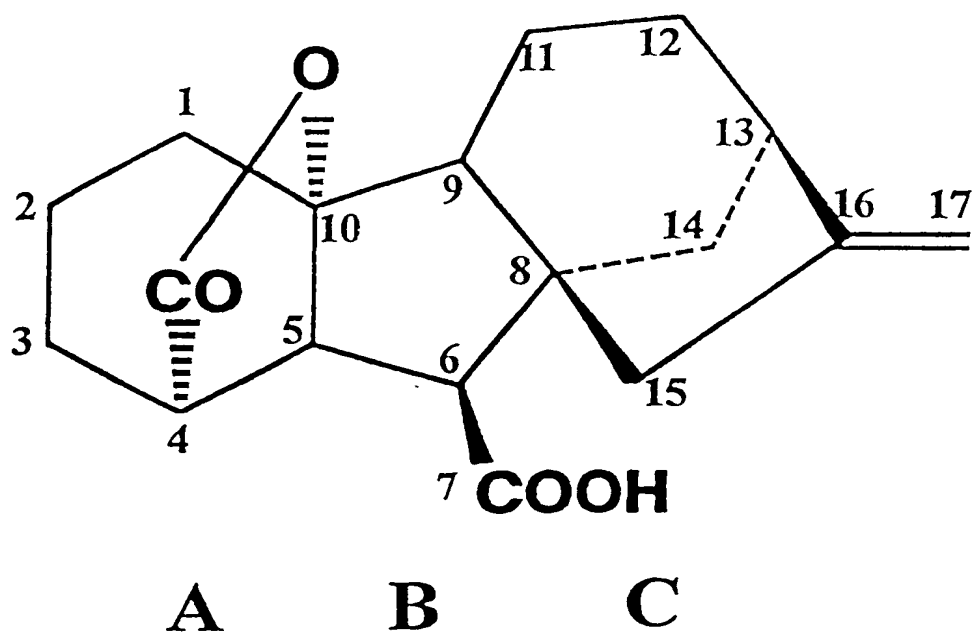
Figure 4a

ACGCGTCCGGAAGCCGGCGGGAGCAGCGGCGGCGGGAGCAGCGCCGATATGGG
GTCGTGCAAGGACAAGGTGATGGCGGGGGCGGCGGGGGAGGAGGAGGACGTCT
ACGAGCTGCTGGCGGCGCTCGGGTACAAGGTGCGGTCGTCCGACATGGCCGACG
TCGCGCAGAANCTGGAGCAGCTGGAGATGGCCATGGGGATGGGCGGCGTGAGCG
CCCCCGGCGCCGCGGATGACGGGTTCGTGTCGCACCTGGCCACGGACACCGTGC
ACTACAACCCCTCGGACCTCTCCTCCTGGGTTCNGAGAGCATGCTTTCGGAGTTA
AAGGCGCCGTTGCCCCTTATCCCGCCAGGCGCCGCCGGGCTGCCCGCCATGCTTT
CCAACTTCGTCCACTGTCACCGGCGGCGGTGGTAGCGGCTTCTTTGAANTCCCAG
CCGCTGCCGANTCGTCGAGTAGCACNTACGCCCTCAGGCCGATCTCCTTACCGGT
GGTGGCGACGGCTGACCCGTCGGCTGCTGACTCGGCGAGGGACACCAAGCGGAT
GCGCACTGGCGGCGGCAGCACGTCGTCGTCCTCATCGTCGTCTTCCTCTCTGGGC
GGTGGGGCCTCGCGGGGCTCTGTGGTGGAGGCTGCTCCGCCGGCGACGCAAGGG
GCCGCGGCGGCGAATGCGCCCGCCGTGCCGGTTGTGGTGGTTGACACGCAGGAG
GCTGGNATCGGGCCTGGTGC

Figure 4b

Protein sequence alignment of Wheat, Rice, and Gai sequences.

Figure 5

A　　　　B　　　　C

Figure 6a

GTCGACCCACGCGTCCGGAAGCCGGCGGGAGCAGCGGCGGCGGGAGCAGCGCC
GATATGGGGTCGTGCAAGGACAAGGTGATGGCGGGGGCGGCGGGGGAGGAGGA
GGACGTCGACGAGCTGCTGGCGGCGCTCGGGTACAAGGTGCGGTCGTCCGACAT
GGCCGACGTCGCGCAGAAGCTGGAGCAGCTGGAGATGGCCATGGGGATGGGCGG
CGTGAGCGCCCCCGGCGCCGCGGATGACGGGTTCGTGTCGCACCTGGCCACGGA
CACCGTGCACTACAACCCCTCGGACCTCTCCTCCTGGGTCGAGAGCATGCTTTCC
GAGCTCAACGCGCCGCTGCCCCCTATCCCGCCAGCGCCGCCGGCTGCCCGCCATG
CTTCCACCTCGTCCACTGTCACCGGCGGCGGTGGTAGCGGCTTCTTTGAACTCCC
AGCCGCTGCCGACTCGTCGAGTAGCACCTACGCCCTCAGGCCGATCTCCTTACCG
GTGGTGGCGACGGCTGACCCGTCGGCTGCTGACTCGGCGAGGGACACCAAGCGG
ATGCGCACTGGCGGCGGCAGCACGTCGTCGTCCTCATCGTCGTCTTCCTCTCTGG
GCGGTGGGGCCTCGCGGGGCTCTGTGGTGGAGGCTGCTCCGCCGGCGACGCAAG
GGGCCGCGGCGGCGAATGCGCCCGCCGTGCCGGTTGTGGTGGTTGACACGCAGG
AGGCTGGGATCCGGCTGGTGCACGCGTTGCTGGCGTGCGCGGAGGCCGTGCAGC
AGGAGAACTTC

Figure 6b

RPTRPEAGGSSGGGSSADMGSCKDKVMAGAAGEEEDVDELLAALGYKVRSSDMAD
VAQKLEQLEMAMGMGGVSAPGAADDGFVSHLATDTVHYNPSDLSSWVESMLSELN
APLPPIPPAPPAARHASTSSTVTGGGGSGFFELPAAADSSSSTYALRPISLPVVATADPS
AADSARDTKRMRTGGGSTSSSSSSSSSLGGGASRGSVVEAAPPATQGAAAANAPAVP
VVVVDTQEAGIRLVHALLACAEAVQQENF

Figure 7a

GCCAGGAGCTCTGTGGTGGAGGCTGCCCCGCCGGTCGCGGCCGCGGCCAACGCG
ACGCCCGCGCTGCCGGTCGTCGTGGTCGACACGCAGGAGGCCGGGATTCGGCTG
GTGCACGCGCTGCTGGCGTGCGCGGAGGCCGTGCAGCAGGAGAACCTCTCCGCC
GCGGAGGCGCTGGTGAAGCAGATACCCTTGCTGGCCGCGTCCCAGGGCGGCGCG
ATGCGCAAGGTCGCCGCCTACTTCGGCGAGGCCCTCGCCCGCCGCGTCTTCCGCT
TCCGCCCGCAGCCGGACAGCTCCCTCCTCGACGCCGCCTTCGCCGACCTCCTCCA
CGCGCACTTCTACGAGTCCTGCCCCTACCTCAAGTTCGCGCACTTCACCGCCAAC
CAGGCCATCCTGGAGGCGTTCGCCGGCTGCCGCCGCGTGCACGTCGTCGACTTCG
GCATCAAGCAGGGGATGCAGTGGCCCGCACTTCTCCAGGCCCTCGCCCTCCGTCC
CGGCGGCCCTCCCTCGTTCCGCCTCACCGGCGTCGGCCCCCCGCAGCCGGACGAG
ACCGACGCCCTGCAGCAGGTGGGCTGGAAGCTCGCCCAGTTCGCGCACACCATC
CGCGTCGACTTCCAGTACCGCGGCCTCGTCGCCGCCACGCTCGCGGACCTGGAGC
CGTTCATGCTGCAGCCGGAGGGCGAGGAGGACCCGAACGAGGAGCCCGAGGTAA
TCGCCGTCAACTCAGTCTTCGAGATGCACCGGCTGCTCGCGCAGCCCGGCGCCCT
GGAGAAGGTCCTGGGCACCGTGCGCGCCGTGCGGCCCAGGATCGTCACCGTGGT
GGAGCAGGAGGCGAATCACAACTCCGGCACATTCCTGGACCGCTTCACCGAGTC
TCTGCACTACTACTCCACCATGTTCGATTCCCTCGAGGGCGGCAGCTCCGGCGGC
GGCCCATCCGAAGTCTCATCGGGGGCTGCTGCTGCTCCTGCCGCCGCCGGCACGG
ACCAGGTCATGTCCGAGGTGTACCTCGGCCGGCAGATCTGCAACGTGGTGGCCTG
CGAGGGGGCGGAGCGCACAGAGCGCCACGAGACGCTGGGCCAGTGGCGGAACC
GGCTGGGCAACGCCGGGTTCGAGACCGTCCACCTGGGCTCCAATGCCTACAAGC
AGGCGAGCACGCTGCTGGCGCTCTTCGCCGGCGGCGACGGCTACAAGGTGGAGG
AGAAGGAAGGCTGCCTGACGCTGGGGTGGCACACGCGCCCGCTGATCGCCACCT
CGGCATGGCGCCTGGCCGGGCCGTGATCTCGCGAGTTTTGAACGCTGTAAGTACA
CATCGTGAGCATGGAGGACAACACAGCCCCGGCGGCCGCCCCGGCTCTCCGGCG
AACGCACGCACGCACGCACTTGAAGAAGAAGAAGCTAAATGTCATGTCAGTGAG
CGCTGAATTGCAGCGACCGGCTACGATCGATCGGGCTACGGGTGGTTCCGTCCGT
CTGGCGTGAAGAGGTGGATGGACGACGAACTCCGAGCCGACCACCACCGGCATG
TAGTAATGTAATCCCTTCTTCGTTCCCAGTTCTCCACCGCCTCCATGATCACCCGT
AAAACTCCTAAGCCCTATTATTACTACTATTATGTTTAAATGTCTATTATTGCTAT
GTGTAATTCCTCCAACCGCTCATATCAAAATAAGCACGGGCCGGAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

Figure 7b

ARSSVVEAAPPVAAAANATPALPVVVVDTQEAGIRLVHALLACAEAVQQENLSAAE
ALVKQIPLLAASQGGAMRKVAAYFGEALARRVFRFRPQPDSSLLDAAFADLLHAHF
YESCPYLKFAHFTANQAILEAFAGCRRVHVVDFGIKQGMQWPALLQALALRPGGPPS
FRLTGVGPPQPDETDALQQVGWKLAQFAHTIRVDFQYRGLVAATLADLEPFMLQPE
GEEDPNEEPEVIAVNSVFEMHRLLAQPGALEKVLGTVRAVRPRIVTVVEQEANHNSG
TFLDRFTESLHYYSTMFDSLEGGSSGGGPSEVSSGAAAAPAAAGTDQVMSEVYLGR
QICNVVACEGAERTERHETLGQWRNRLGNAGFETVHLGSNAYKQASTLLALFAGGD
GYKVEEKEGCLTLGWHTRPLIATSAWRLAGP

Figure 8a

ATAGAGAGGCGAGGTAGCTCGCGGATCATGAAGCGGGAGTACCAGGACGCCGG
AGGGAGCGGCGGCGGCGGTGGCGGCATGGGCTCGTCCGAGGACAAGATGATGGT
GTCGGCGGCGGCGGGGGAGGGGGAGGAGGTGGACGAGCTGCTGGCGGCGCTCG
GGTACAAGGTGCGCGCCTCCGACATGGCGGACGTGGCGCAGAAGCTGGAGCAGC
TCGAGATGGCCATGGGGATGGGCGGCGTGGGCGCCGGCGCCGCCCCCGACGACA
GCTTCGCCACCCACCTCGCCACGGACACCGTGCACTACAACCCCACCGACCTGTC
GTCTTGGGTCGAGAGCATGCTGTCGGAGCTCAACGCGCCGCCGCCGCCCCTCCCG
CCCGCCCCGCAGCTCAACGCCTCCACCTCCTCCACCGTCACGGGCAGCGGCGGCT
ACTTCGATCTCCCGCCCTCCGTCGACTCCTCCAGCAGCATCTACGCGCTGCGGCC
GATCCCCTCCCCGGCCGGCGCGACGGCGCCGGCCGACCTGTCCGCCGACTCCGTG
CGGGATCCCAAGCGGATGCGCACTGGCGGGAGCAGCACCTCGTCGTCATCCTCCT
CCTCGTCGTCTCTCGGTGGGGGCGCCAGGAGCTCTGTGGTGGAGGCTGCCCCGCC
GGTCGCGGCCGCGGCCAACGCGACGCCCGCGCTGCCGGTCGTCGTGGTCGACAC
GCAGGAGGCCGGGATTCGGCTGGTGCACGCGCTGCTGGCGTGCGCGGAGGCCGT
GCAGCAGGAGAACCTCTCCGCCGCGGAGGCGCTGGTGAAGCAGATACCCTTGCT
GGCCGCGTCCCAGGGCGGCGCGATGCGCAAGGTCGCCGCCTACTTCGGCGAGGC
CCTCGCCCGCCGCGTCTTCCGCTTCCGCCCGCAGCCGGACAGCTCCCTCCTCGAC
GCCGCCTTCGCCGACCTCCTCCACGCGCACTTCTACGAGTCCTGCCCCTACCTCAA
GTTCGCGCACTTCACCGCCAACCAGGCCATCCTGGAGGCGTTCGCCGGCTGCCGC
CGCGTGCACGTCGTCGACTTCGGCATCAAGCAGGGGATGCAGTGGCCCGCACTTC
TCCAGGCCCTCGCCCTCCGTCCCGGCGGCCCTCCCTCGTTCCGCCTCACCGGCGTC
GGCCCCCCGCAGCCGGACGAGACCGACGCCCTGCAGCAGGTGGGCTGGAAGCTC
GCCCAGTTCGCGCACACCATCCGCGTCGACTTCCAGTACCGCGGCCTCGTCGCCG
CCACGCTCGCGGACCTGGAGCCGTTCATGCTGCAGCCGGAGGGCGAGGAAGACC
CGAACGAGGAGCCCGAGGTAATCGCCGTCAACTCAGTCTTCGAGATGCACCGGC
TGCTCGCGCAGCCCGGCGCCCTGGAGAAGGTCCTGGGCACCGTGCGCGCCGTGC
GGCCCAGGATCGTCACCGTGGTGGAGCAGGAGGCGAATCACAACTCCGGCACAT
TCCTGGACCGCTTCACCGAGTCTCTGCACTACTACTCCACCATGTTCGATTCCCTC
GAGGGCGGCAGCTCCGGCGGCGGCCCATCCGAAGTCTCATCGGGGGCTGCTGCT
GCTCCTGCCGCCGCCGGCACGGACCAGGTCATGTCCGAGGTGTACCTCGGCCGGC
AGATCTGCAACGTGGTGGCCTGCGAGGGGGCGGAGCGCACAGAGCGCCACGAGA
CGCTGGGCCAGTGGCGGAACCGGCTGGGCAACGCCGGGTTCGAGACCGTCCACC
TGGGCTCCAATGCCTACAAGCAGGCGAGCACGCTGCTGGCGCTCTTCGCCGGCGG
CGACGGCTACAAGGTGGAGGAGAAGGAAGGCTGCCTGACGCTGGGGTGGCACAC
GCGCCCGCTGATCGCCACCTCGGCATGGCGCCTGGCCGGGCCGTGATCTCGCGAG
TTTGAACGCTGTAAGTACACATCGTGAGCATGGAGGACAACACAGCCCCGGCG
GCCGCCCCGGCTCTCCGGCGAACGCACGCACGCACGCACTTGAAGAAGAAGAAG
CTAAATGTCATGTCAGTGAGCGCTGAATTGCAGCGACCGGCTACGATCGATCGGG
CTACGGGTGGTTCCGTCCGTCTGGCGTGAAGAGGTGGATGGACGACGAACTCCG

Figure 8b

MKREYQDAGGSGGGGGGMGSSEDKMMVSAAAGEGEEVDELLAALGYKVRASDM
ADVAQKLEQLEMAMGMGGVGAGAAPDDSFATHLATDTVHYNPTDLSSWVESMLS
ELNAPPPPLPPAPQLNASTSSTVTGSGGYFDLPPSVDSSSSIYALRPIPSPAGATAPADL
SADSVRDPKRMRTGGSSTSSSSSSSSSSLGGGARSSVVEAAPPVAAAANATPALPVVV
VDTQEAGIRLVHALLACAEAVQQENLSAAEALVKQIPLLAASQGGAMRKVAAYFGE
ALARRVFRFRPQPDSSLLDAAFADLLHAHFYESCPYLKFAHFTANQAILEAFAGCRR
VHVVDFGIKQGMQWPALLQALALRPGGPPSFRLTGVGPPQPDETDALQQVGWKLA
QFAHTIRVDFQYRGLVAATLADLEPFMLQPEGEEDPNEEPEVIAVNSVFEMHRLLAQ
PGALEKVLGTVRAVRPRIVTVVEQEANHNSGTFLDRFTESLHYYSTMFDSLEGGSSG
GGPSEVSSGAAAAPAAAGTDQVMSEVYLGRQICNVVACEGAERTERHETLGQWRN
RLGNAGFETVHLGSNAYKQASTLLALFAGGDGYKVEEKEGCLTLGWHTRPLIATSA
WRLAGP

Figure 9a

TTTCGCCTGCCGCTGCTATTAATAATTGCCTTCTTGGTTTCCCCGTTTTCGCCCCAG
CCGCTTCCCCCCTCCCCTACCCTTTCCTTCCCCACTCGCACTTCCCAACCCTGGAT
CCAAATCCCAAGCTATCCCAGAACCGAAACCGAGGCGCGCAAGCCATTATTAGC
TGGCTAGCTAGGCCTGTAGCTCCGAAATCATGAAGCGCGAGTACCAAGACGCCG
GCGGGAGTGGCGGCGACATGGGCTCCTCCAAGGACAAGATGATGGCGGCGGCGG
CGGGAGCAGGGGAACAGGAGGAGGAGGACGTGGATGAGCTGCTGGCCGCGCTC
GGGTACAAGGTGCGTTCGTCGGATATGGCGGACGTCGCGCAGAAGCTGGAGCAG
CTCGAGATGGCCATGGGGATGGGCGGCGTGGGCGGCGCCGGCGCTACCGCTGAT
GACGGGTTCGTGTCGCACCTCGCCACGGACACCGTGCACTACAATCCCTCCGACC
TGTCGTCCTGGGTCGAGAGCATGCTGTCCGAGCTCAACGCGCCCCCAGCGCCGCT
CCCGCCCGCGACGCCGGCCCCAAGGCTCGCGTCCACATCGTCCACCGTCACAAGT
GGCGCCGCCGCCGGTGCTGGCTACTTCGATCTCCCGCCCGCCGTGGACTCGTCCA
GCAGTACCTACGCTCTGAAGCCGATCCCCTCGCCGGTGGCGGCGCCGTCGGCCGA
CCCGTCCACGGACTCGGCGCGGGAGCCCAAGCGGATGAGGACTGGCGGCGGCAG
CACGTCGTCCTCCTCTTCCTCGTCGTCATCCATGGATGGCGGTCGCACTAGGAGCT
CCGTGGTCGAAGCTGCGCCGCCGGCGACGCAAGCATCCGCGGCGGCCAACGGGC
CCGCGGTGCCGGTGGTGGTGGTGGACACGCAGGAGGCCGGGATCCGGCTCGTGC
ACGCGCTGCTGGCGTGCGCGGAGGCCGTGCAGCAGGAGAACTTCTCTGCGGCGG
AGGCGCTGGTCAAGCAGATCCCCATGCTGGCCTCGTCGCAGGGCGGTGCCATGC
GCAAGGTCGCCGCCTACTTCGGCGAGGCGCTTGCCCGCCGCGTGTATCGCTTCCG
CCCGCCACCGGACAGCTCCCTCCTCGACGCCGCCTTCGCCGACCTCTTGCACGCG
CACTTCTACGAGTCCTGCCCCTACCTGAAGTTCGCCCACTTCACCGCGAACCAGG
CCATCCTCGAGGCCTTCGCCGGCTGCCGCCGCGTCCACGTCGTCGACTTCGGCAT
CAAGCAGGGGATGCAGTGGCCGGCTCTTCTCCAGGCCCTCGCCCTCCGCCCTGGC
GGCCCCCCGTCGTTCCGGCTCACCGGCGTCGGGCCGCCGCAGCCCGACGAGACC
GACGCCTTGCAGCAGGTGGGCTGGAAACTTGCCCAGTTCGCGCACACCATCCGCG
TGGACTTCCAGTACCGTGGCCTCGTCGCGGCCACGCTCGCCGACCTGGAGCCGTT
CATGCTGCAACCGGAGGGCGATGACACGGATGACGAGCCCGAGGTGATCGCCGT
GAACTCCGTGTTCGAGCTGCACCGGCTTCTTGCGCAGCCCGGTGCCCTCGAGAAG
GTCCTGGGCACGGTGCGCGCGGTGCGGCCGAGGATCGTGACCGTGGTCGAGCAG
GAGGCCAACCACAACTCCGGCACGTTCCTCGACCGCTTCACCGAGTCGCTGCACT
ACTACTCCACCATGTTCGATTCTCTCGAGGGCGCCGGCGCCGGCTCCGGCCAGTC
CACCGACGCCTCCCCGGCCGCGGCCGGCGGCACGGACCAGGTCATGTCGGAGGT
GTACCTCGGCCGGCAGATCTGCAACGTGGTGGCGTGCGAGGGCGCGGAGCGCAC
GGAGCGCCACGAGACGCTGGGCCAGTGGCGCAGCCGCCTCGGCGGCTCCGGGTT
CGCGCCCGTGCACCTGGGCTCCAATGCCTACAAGCAGGCGAGCACGCTGCTGGC
GCTCTTCGCCGGCGGCGACGGGTACAGGGTGGAGGAGAAGGACGGGTGCCTGAC
CCTGGGGTGGCATACGCGCCCGCTCATCGCCACCTCGGCGTGGCGCGTCGCCGCC
GCCGCCGCTCCGTGATCAGGGAGGGGTGGTTGGGGCTTCTGGACGCCGATCAAG
GCACACGTACGTCCCCTGGCATGGCGCACCCTCCCTCGAGCTCGCCGGCACGGGT
GAAGCTACCCGGGGGATCCACTAATTCTAAAACGGCCCCACCGCGGTGGAACTC
CACCTTTTGTTCCCTTTA

Figure 9b

MKREYQDAGGSGGDMGSSKDKMMAAAAGAGEQEEEDVDELLAALGYKVRSSDM
ADVAQKLEQLEMAMGMGGVGGAGATADDGFVSHLATDTVHYNPSDLSSWVESML
SELNAPPAPLPPATPAPRLASTSSTVTSGAAAGAGYFDLPPAVDSSSSTYALKPIPSPV
AAPSADPSTDSAREPKRMRTGGGSTSSSSSSSSSSMDGGRTRSSVVEAAPPATQASAAA
NGPAVPVVVVDTQEAGIRLVHALLACAEAVQQENFSAAEALVKQIPMLASSQGGAM
RKVAAYFGEALARRVYRFRPPPDSSLLDAAFADLLHAHFYESCPYLKFAHFTANQAI
LEAFAGCRRVHVVDFGIKQGMQWPALLQALALRPGGPPSFRLTGVGPPQPDETDAL
QQVGWKLAQFAHTIRVDFQYRGLVAATLADLEPFMLQPEGDDTDDEPEVIAVNSVF
ELHRLLAQPGALEKVLGTVRAVRPRIVTVVEQEANHNSGTFLDRFTESLHYYSTMFD
SLEGAGAGSGQSTDASPAAAGGTDQVMSEVYLGRQICNVVACEGAERTERHETLGQ
WRSRLGGSGFAPVHLGSNAYKQASTLLALFAGGDGYRVEEKDGCLTLGWHTRPLIA
TSAWRVAAAAP

Figure 10

Figure 10 (Continued)

maiz-fin   RIVTVVEQEA NHNSGTFLDR FTESLHYYST MFDSLEGAGA GSGQSTDASP A.....AAGGT   529
rht-fina   RIVTVVEQEA NHNSGTFLDR FTESLHYYST MFDSLEGGSS GGGPSEVSSG AAAPAAAGT   525
rice-fin   256
gai   EIFTVVEQES NHNSPIFLDR FTESLHYYST FDSLEGVPS GQ.......   434

maiz-fin   DQVMSEVYLG RQICNVVACE GAERTERHET LGQWRSRLGG SGFAPVHLGS NAYKQASTLL   589
rht-fina   DQVMSEVYLG RQICNVVACE GAERTERHET LGQWRNRLGN AGFETVHLGS NAYKQASTLL   585
rice-fin   256
gai   DKVMSEVYLG RQICNVVACD GPDRVERHET SQWRNRREGS AGFAAAHIGS NAHKQASMLL   494

maiz-fin   ALFAGGDGYR VEEKDGCLTL GWHTRPLIAT SAMRVAAAAA P   630
rht-fina   ALFAGGDGYR VEEKDGCLTL GWHTRPLIAT SAMRLAGP... .   623
rice-fin   256
gai   ALFNGGDGYR VEESDGCLML GWHTRPLIAT SAWKLSTN.. .   532

Figure 11a

TACCAAGACGCCGGCGGGAGTGGCGGCGACATGGGCTCCTCCAAGGACAAGATG
ATGGCGGCGGCGGCGGGAGCAGGGGAACAGGAGGAGGAGGACGTGGATGAGCT
GCTGGCCGCGCTCGGGTACAAGGTGCGTTCGTCGGATATGGCGGGGCTGGAGCA
GCTCGAGATGGCCATGGGGATGGGCGGCGTGGGCGGCGCCGGCGCTACCGCTGA
TGACGGGTTCGTGTCGCACCTCGCCACGGACACCGTGCACTACAATCCCTCCGAC
CTGTCGTCCTGGGTCGAGAGCATGCTGTCCGA

Figure 11b

YQDAGGSGGDMGSSKDKMMAAAAGAGEQEEEDVDELLAALGYKVRSSDMAGLEQ
LEMAMGMGGVGGAGATADDGFVSHLATDTVHYNPSDLSSWVESMLS

Figure 11c

TCCTCCAAGGACAAGATGATGGCGGCGGCGGCGGGAGCAGGGGAACAGGAGGA
GGAGGACGTGGATGAGCTGCTGGCCGCGCTCGGGTACAAGGTGCGTTCGTCGGA
TATGGCGGACGTCGCGCAGAAGCTGGAGCAGCTCGAGATGGCCATGGGGATGGG
CGGCGTGGGCGGCGCCGGCGCTACCGCTGATGACGGGTTCGTGTCGCACCTGTCG
TCCTGGGTCGAGAGCATGCTGTCCGAGCTCAACGCGCCCCCAGCGCCGCTCCCGC
CCGCGACGCCGGCCCCAAGGCTCGCGTCCACATCGTCCACCGTCACAAGTGGCGC
CGCCGCCGGTGCTGGCTACTTCGATCTCCCGCCCGCCGTGGACTC

Figure 11d

SSKDKMMAAAAGAGEQEEEDVDELLAALGYKVRSSDMADVAQKLEQLEMAMGM
GGVGGAGATADDGFVSHLSSWVESMLSELNAPPAPLPPATPAPRLASTSSTVTSGAA
AGAGYFDLPPAVD

Figure 12a

GCGGCGCTCGGGTACAAGGTGCGCGCCTCCGACATGGCGGACGTGGCGCAGAAG
CTGGAGCAGCTCGAGATGGCCATGGGGATGGGCGGCGTGGGCGCCGGCGCCGCC
CCCGACGACAGCTTCGCCACCCACCTCGCCACGGACACCGTGCACTACAACCCCA
CCGACCTGTCGTCTTGGGTCGAGAGCATGCTGTCGGAGCTCAACGCCTCCACCTC
CTCCACCGTCACGGGCAGCGGCGGCTACTTCGATCTCCCGCCCTCCGTCGACTCC
TCCAGCAGCATCTACGCGCTGCGGCCGATCCCCTCCCCGGCCGGCGCGACGGCGC
CGGCCGACCTGTCCGCCGACTCCGTGCGGGATCCCAAGCGGATGCGCACTGGCG
GGAGCAGCACCTCGTCGTCATCCTCCTCCTCGTC

Figure 12b

AALGYKVRASDMADVAQKLEQLEMAMGMGGVGAGAAPDDSFATHLATDTVHYN
PTDLSSWVESMLSELNASTSSTVTGSGGYFDLPPSVDSSSSIYALRPIPSPAGATAPAD
LSADSVRDPKRMRTGGSSTSSSSSSS

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6   C12N15/29   C12N15/82   C12Q1/68   A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6   C12N   C12Q   A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

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X	SASAKI, T., ET AL.: "Rice cDNA, partial sequence (S0803_1A)" EMBL ACCESSION NO.D39460.13 November 1994, XP002088385 see the whole document	6,8,9, 12-15, 18,23,30
	—/—	

[X] Further documents are listed in the continuation of box C.		[X] Patent family members are listed in annex.

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

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Date of the actual completion of the international search	Date of mailing of the international search report
17 December 1998	12/01/1999

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

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Int   tional Application No

PCT/GB 98/02383

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Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
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